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BATCH ANAEROBIC BIOLOGICAL TREATMENT OF REFINERY SOUR WATER

by

IAN MACKENZIE

(C)

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ABSTRACT

The objective of this research was to assess the treatability of refinery sour water by anaerobic biological processes.

Four sour water samples from three local refineries were obtained for this purpose. Batch anaerobic cultures containing various dilutions of the individual sour water samples were monitored for methane production.

All of the samples were pretreated before incubation. Nitrogen stripping the sample was the most commonly used pretreatment option. Pretreating the sample by batch adsorption with activated carbon and boiling the sample were also practiced.

The results indicated that the major sour water organic, phenol, can be degraded at various sample concentrations (10% to 50% sample concentration by volume), depending upon the type of pretreatment given the sample. Inhibition of methane generation, which occurred more readily in the absence of boiling pretreatment, was found to be caused by inorganic constituents within the sour water. The phenol degrading bacteria were found to be more sensitive to toxicants than the methane producing acetate degraders.

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LIST OF ABBREVIATIONS USED IN TEXT

A.C.	activated carbon
BOD	biochemical oxygen demand
BOD_5	five day biochemical oxygen demand
COD	chemical oxygen demand
DOC	dissolved organic carbon
pH	hydrogen ion concentration
SS	suspended solids
TSS	total suspended solids
TOC	total organic carbon

1. INTRODUCTION

Sour water is a highly contaminated waste stream within a petroleum refinery and it requires intensive treatment before reuse or discharge into a watercourse.

If sour water is to receive biological treatment, it is first pretreated, usually by steam stripping, to remove inorganic pollutants such as sulphide, ammonia, and cyanide. An aerobic biological treatment system, such as an activated sludge unit, is often used to reduce the remaining organic constituents to acceptable discharge levels.

Aerobic biological treatment has generally proven to be effective in reducing the organic load to acceptable levels, but the long hydraulic retention times and the associated intensive energy requirements, make it an expensive endeavour.

An anaerobic biological pretreatment step has the potential of lowering the total influent organic load to an aerobic biological treatment system. Other advantages associated with successfully operating anaerobic treatment systems are a low sludge generation rate, low energy requirements, and methane fuel production.

Sour water is a good candidate for anaerobic treatment because it has a high phenol concentration. It is well documented that phenol is anaerobically decomposable to methane and carbon dioxide. Therefore, sour water contains a suitable substrate for the anaerobic microorganisms. However, the question is, can these microorganisms function

effectively in the presence of other potentially toxic organic and inorganic constituents?

The overall objective of this research was to answer this question and also to seek a means of enhancing the degradability of sour water through appropriate non-biological pretreatment methods.

Four sour water samples from three Edmonton refineries were obtained for this research. A serum bottle modification of the Hungate technique (Hungate 1950) was used to determine the treatability of the sour wastewater. Each serum bottle served as a batch anaerobic reactor into which varying proportions of pretreated sour water was added for incubation. Methane production was adopted as an indicator of organic stabilization.

2. SOUR WATER CHARACTERIZATION AND TREATMENT

2.1 CHARACTERIZATION

2.1.1 Introduction

Refinery wastewater containing hydrogen sulphide is often referred to as sour or foul water. Other accompanying constituents such as ammonia, organic nitrogen compounds and phenolics also contribute to the foul nature of this highly toxic wastewater.

Sour water originates from processes that allow contact between steam or process water and the hydrocarbon phase. Some of the major contributing operations ranked in order of importance, are:

1. catalytic and thermal cracking
2. distillation
3. crude desalting
4. hydrocracking
5. alkylation
6. hydrotreating
7. reforming
8. polymerization

The major source of sulphide and other contaminants is the

condensed steam from catalytic or thermal cracking operations. Steam is used here and in distillation columns as a stripping agent and diluent to reduce the hydrocarbon partial pressure. Condensation of steam and the hydrocarbon liquids occurs simultaneously in the presence of a hydrocarbon vapour phase containing H_2S. After condensation and separation from the hydrocarbon layer, a sour condensate is produced containing NH_3, H_2S, oil, and soluble organic acids.

Typical ranges of the major contaminants in sour water are;

H_2S	275 - 11000 mg/L
NH_3	100 - 7000 mg/L
phenol	100 - 1000 mg/L
cyanide	18 - 144 mg/L

2.1.2 Inorganic Contaminants

Quantitatively, the most significant inorganic compounds in untreated sour water are H_2S, NH_3 and cyanide. Other constituents such as ions and heavy metals may also be found in significant quantities, depending on the contributing unit processes; for instance, ions like chlorides and sodium may be found in high concentrations (> 1000 mg/L) especially in sour waters originating from desalting operations.

Wilson and Roberts (1981) found mean levels of heavy metals in sour waters ranging from 1 mg/L for mercury and

cadmium, 20 - 325 mg/L for copper, and 100 - 260 mg/L for zinc. High lead levels, 10 - 140 mg/L, may also be found when it is used as a gasoline additive.

Some additional metals found in general process wastewater are aluminum, arsenic, chromium, cobalt, iron, nickel, and vanadium (U.S. EPA 1973). Major sources of these metals are the crude oil and corrosion products.

2.1.3 Organic Contaminants

Contact of the process water or steam with the crude feedstock or hydrocarbon phase will result in significant organic contamination of the water.

Crude oil contains three main categories of organics: paraffins (alkanes), naphthenes (cycloalkanes), and aromatics. Some hydrocarbons from these three groups will be extracted into the process water.

Another organic class, olefins (unsaturated hydrocarbons), are formed during processing and may be extracted into the water phase. Oxygenated compounds, such as phenolics, are formed during distillation and cracking operations within a high temperature and pressure environment.

For the purposes of treatment evaluation, a complete characterization of all organics in refinery sour water is both difficult and questionable in value. Such analysis would be difficult – because many hundreds of organics would be present, and questionable – because of the variable and

diverse character of sour waters. Different refineries and different crude sources produce widely varying wastewater parameters.

Nevertheless, a couple of studies have attempted a comprehensive analysis of sour water (CONCAWE 1982, and American Petroleum Institute 1978). These studies were mainly inspired by a recent recognition that some toxic or persistent organic compounds may bioaccumulate in receiving water courses. An important finding (CONCAWE 1982) was that the organics in sour waters from different crude sources and refineries vary much less qualitatively than quantitatively.

2.1.3.1 Phenolics

Phenolics are significant because of their toxic and carcinogenic properties. Bad tastes and odours from phenolics can be detected in water at the parts per billion level; moreover, these objectionable characteristics can be amplified when the phenolics are combined with chlorine.

The concentration of phenolics in sour condensate depends on temperature, pH, and their solubility in water; for instance, thiophenolics (such as phenyl mercaptans) are very insoluble in water and will remain almost completely in the hydrocarbon phase.

The major phenolics in sour water are monohydrics, such as phenol, o-, m-, and p-cresols, various xylenols, ethylphenolics, and dihydrics like catechol and

resorcinol (Beychok 1967). However, phenol is the predominant phenolic compound found.

Beychok (1967) conducted a detailed study of various sour condensates to determine the relative distribution of phenolics in the hydrocarbon and aqueous phases. His conclusions were:

1. The amount of phenolics in the aqueous phase is related to the amount of phenolics in the hydrocarbon phase.
2. The type of phenolics present affects the equilibrium distribution because the solubility in water varies for the different monohydric phenolics.
3. The type of hydrocarbons present (saturates, olefins, or aromatics) will also affect the equilibrium distribution of phenolics between the hydrocarbon liquid phase and the aqueous phase.
4. Both the amount and type of monohydric phenolics present in the hydrocarbon phase will depend on the hydrocarbon phase boiling range. The type of process (catalytic cracking, thermal cracking, etc.) also affects the amount of phenolics present in the hydrocarbon.
5. The pH of the aqueous phase affects the equilibrium distribution because a higher pH will increase the ionization of the weakly acidic phenolics and hence cause more phenolics to be extracted into the aqueous phase.

The objective of sour water treatment is the reduction of the previously mentioned contaminants to non-toxic levels. Sulphide and ammonia levels are usually reduced by steam stripping pretreatment prior to biological treatment. Secondary biological treatment is optimized for organics, especially phenolics removal.

2.2 TREATMENT

2.2.1 Pretreatment of Sour Waters

Before sour water can be biologically treated it must be pretreated to lower sulfides, ammonia, and cyanides to levels that can be tolerated by the microorganisms.

High temperature (38 - 132°C) and pressure (7 - 345 kPa) stripping columns are commonly employed for this purpose. Depending on the design, about 1 - 4 kg steam per liter of sour water stripped is required (Grant 1974). Often, the pH is lowered to about 6.5 by acid addition to facilitate sulfide conversion to gaseous H_2S. By lowering the pH, ammonia is fixed in the ionic form with the salt of a strong acid, effectively preventing its escape as a gas. When ammonia removal is desired the pH is appropriately adjusted (pH 10) to allow ammonia removal as a gas.

Strippers can be designed to achieve almost any degree of reduction of any volatile contaminant, but when multiple contaminants are present, which is usually the case, the

problem becomes complex and expensive.

Under normal stripping column conditions, sulphide removals approaching 100% can be achieved. Ammonia removal, when conditions are optimized for sulfide reduction, are negligible. Phenol removals as high as 35% have been attained (Beychok 1967), but for design purposes, an estimate of 20% phenol reduction is commonly used.

Another system used for sulphide removal is a sulphide oxidizer. With this system, sour waters, including spent caustics, can be processed for the oxidation of sulphides to thiosulphates and sulphates. The process uses air as the oxidizing agent and pressures and temperatures in the ranges of 70 - 350 kPa and 104 - 138°C respectively. Mercaptans are reduced by a combination of oxidation and stripping.

Chemical oxidation or precipitation of sulphides is costly and not in common use. In newer refineries sour water stripper bottoms may be used as wash water for desalting operations. This process partitions phenolics back into the hydrocarbon phase.

2.2.2 Conventional Biological Treatment

After pretreatment, the sour water requires secondary treatment to further reduce organic contaminant concentrations. Biological treatment of refinery sour water is presently the most cost effective means of achieving this reduction.

The usual parameters of interest for determining the efficiency of treatment are phenolics, COD or BOD_5, TOC, sulphide, ammonia, SS, and pH. The ability of biological systems to achieve significant reductions of these parameters is generally unequaled.

The most frequently used biological treatment system in refineries today is the complete-mix activated sludge unit. Other commonly used systems are trickling filters and oxidation ponds.

Aerobic biological treatment can be expected to remove lighter aromatic and paraffinic hydrocarbons, phenolics, carboxylic acids, cyanides, and sulphides. Some specific organic compound reductions afforded by various biological treatment processes have been investigated by CONCAWE (1982), and Raphaelian and Harrison (1978).

Aerobic biological treatment for phenol destruction is effective over a wide range of phenol levels, ranging from 7 to 10 mg/L to several thousand milligrams per liter. Many treatment plants report final phenol effluent concentrations of about 0.1 mg/L for raw influent waste concentrations of 1000 mg/L (Kostenbader and Flecksteiner 1969).

Typical final effluent parameters after activated sludge treatment are presented in Table 1 (CONCAWE 1982).

Table 1. Typical effluent parameters from an activated sludge treatment plant (CONCAWE 1982).

PARAMETER	MAXIMUM CONCENTRATION IN EFFLUENT (mg/L)
SS	30
oil	5
sulphides	undetectable
phenolics	0.5
BOD_5	25

Successful operation of a biological treatment system depends primarily on the plant's ability to ensure stable influent wastewater parameters such as pH, temperature, oxygen levels, oil or SS. Toxic slugs of phenolics, H_2S, or other inorganic toxicants must also be avoided.

Activated carbon is often added as a supplement to an activated sludge unit. The advantage of using activated carbon is its ability to reduce levels of toxic and non-biodegradable refractory organics. Activated carbon has the effect of dampening toxic surges and removing a portion of the organics that would otherwise remain in the effluent.

Anaerobic biological treatment is suggested as an alternative or additional form of treatment because of several potential attributes. Compared to an aerobic treatment system, a well operating anaerobic treatment system will produce low sludge volumes and require less energy. Additionally, an anaerobic treatment system could reduce the organic load to a receiving aerobic system. A criterion to assess the anaerobic treatability of petroleum refinery wastewater would be the systems ability to provide a reasonable rate of microbial degradation of the organics. This is a function of both the type and concentration of organics present and the concentration of the inhibiting compounds in the wastewater to be treated.

Chemical oxidation, using agents such as H_2O_2, ozone, or chlorine dioxide has been used, but is usually only economical when used to dampen toxic surges in equalization tanks prior to biological treatment.

3. ANAEROBIC TREATMENT

3.1 INTRODUCTION

The first step that should be taken to assess the biological treatability of an industrial wastewater is to determine whether the predominant organics will provide a suitable substrate. As mentioned before, the major organics in sour water are phenolics and some of these phenolics are degradable. However, a few questions arise: what concentrations, types, and combinations of phenolics can be degraded; and can the microorganisms function in the presence of other toxic components present in the sour water? The question regarding degradable concentrations and combinations of phenolics is addressed by a review of the research on pure phenolics (section 3.4) and anaerobic research on similar phenolic wastewaters (section 3.5). Possible interference of toxic components in sour water with anaerobic biological treatment is also reviewed (section 3.6).

Finally, pretreatment alternatives, some of which are related to the anaerobic methodology used for shale-oil retort wastewaters are reviewed (section 3.7).

First an introduction to the current status and theory of anaerobic bio-technology is presented.

3.2 ANAEROBIC THEORY

The present theory of anaerobic digestion recognizes four groups of bacteria, with each group being interdependent on the other (Speece 1983).

The first group, the acid forming bacteria, breakdown complex organic matter into a variety of products including acetic, butyric, or propionic acids, H_2 and CO_2. The products (except acetic acid and H_2) from the acid formers are then processed by a second group of non-methanogens, the acetogenic bacteria. The major products from these bacteria are acetic acid, H_2 and CO_2. Acetic acid, CO_2, and H_2 are directly utilized and converted to CH_4 by the acetoclastic methane bacteria and the H_2-utilizing methane bacteria.

The acetoclastic methane bacteria co-operate with the acid forming bacteria to control the concentration of acetic acid and hence the pH value of the solution. This can best be explained with an example.

When surge loads occur, the acid forming bacteria respond by producing large quantities of acetic acid that cannot be processed at a fast enough rate by the acetoclastic methane bacteria. This causes a build-up of acetic acid which tends to depress the pH and allow built-up H_2 gas produced by the acid formers to escape from the solution. The H_2 gas produced signals the acid formers to slow down and produce butyric or propionic acid in amounts depending on the trace H_2 gas concentration. This decreases the rate of pH drop and allows the slower growing

acetoclastic methanogens to process the backlog of acetate. The advantage of this response is that each mole of butyric acid produced replaces the production of two moles of acetic acid, which correspondingly reduces produced acidity.

3.3 CURRENT STATUS OF INDUSTRIAL ANAEROBIC TREATMENT

In the past, a number of industrial wastewaters were precluded from anaerobic treatability studies on the basis of anticipated toxicity of substrate organic matter. This perception may have originated because of the inability to provide suitable conditions for the microorganisms. However, if proper attention is paid to process design and microbiological requirements, many of these organic wastewaters can be treated at some concentration. Recent progress in the understanding of the microbiological and biochemical aspects of anaerobic systems has enabled widespread applications for industrial wastewater treatment.

One example has been the recognition of the uniqueness of the acetogens and methanogens in terms of their trace nutrient requirements. In fact, several previously undiscovered trace nutrient requirements have left some past treatability studies open to criticism.

The need for trace levels of iron as a nutrient was recognized several decades ago, but the difficulty of providing adequate iron in solution was not realized until more recently (Speece 1983). The nickel requirement of methanogens is another of their distinct features because

nickel is not essential for the growth of most bacteria. The importance of nickel as a nutrient was probably not discovered until more recently because of its presence in most solutions as an unknown contaminant resulting from the use of stainless steel instruments and vessels. Speece (1983) observed that intentional nickel addition to acetate-utilizing methanogens resulted in higher methane production figures than previously observed.

In addition to these and other significant advances in the understanding of the biochemical and microbiological aspects of anaerobic treatment there has also been development of several new reactor designs. One of the most revolutionary has been the UASB (Upflow Anaerobic Sludge Blanket) reactor developed by Lettinga in 1979. This system, among others, has enabled treatment of relatively dilute wastewaters from the sugar beet and brewing industries. More details of this system and others are presented in Speece (1983), and Mosey (1983).

3.4 THE ANAEROBIC DEGRADATION OF PHENOLS

Some of the first work on the anaerobic degradation of aromatic compounds began with an investigation into the fate of benzoate. Clark and Fina (1952) showed that benzoic acid degradation was possible and observed that 75% of the methane produced resulted from its degradation through some unknown path, while the other 25% resulted from CO_2 conversion.

Since then, several researchers have investigated the degradation of phenolics and aromatics. A summary of some of these research efforts follows.

Tarvin and Buswell (1934) developed a chemical equation that could be used to predict the quantity of methane produced from the anaerobic degradation of organics. Accordingly, anaerobic phenol degradation would yield the following equation:

$$C_6H_6O + 4H_2O = 2.5CO_2 + 3.5CH_4 \quad (1)$$

If 3.5 moles of methane are produced for every mole of phenol then the gas produced will be 58.3% methane. Some of the CO_2 will remain in solution because of its reactions with water and the hydroxide ion to form alkalinity. For instance, microorganisms are capable of deaminating biodegradable protein and releasing ammonia into solution which will react with water as follows:

$$NH_3 + H_2O = NH_4^+ + OH^- \quad (2)$$

and the hydroxide ion released reacts with aqueous carbon dioxide to form bicarbonate and carbonate ions. These bicarbonate and carbonate complexes can reduce the CO_2 reaching the gas phase by as much as 10% (Healy and Young

1979).

Chmielowski et al. (1965) after previously showing that phenol could be anaerobically degraded (reaffirming the work of Tarvin and Buswell 1934), assessed the anaerobic degradability of various mono- and polyhydric phenolics, naphthols, and related aromatic compounds.

They discovered that nine of the eighteen phenolics studied; including phenol, p-cresol, and resorcinol; were convertible to methane and CO_2. Some monohydric phenolics found to be resistant to methane fermentation were o-cresol, m-cresol, and all the xylenols which they tested. The polyhydric phenolics, catechol and hydroquinone, did not undergo decomposition. Catechol is a common intermediate in the aerobic degradation of many phenolic compounds, and because it was found to be anaerobically non-biodegradable the researchers concluded that phenol must be anaerobically decomposed by a different metabolical pathway; although it was later demonstrated that catechol was anaerobically decomposable, their conclusion presuming a different metabolic pathway was correct.

Healy and Young (1978) demonstrated that catechol as well as phenol were amenable to anaerobic degradation, although acclimation times of 32 and 18 days respectively were required before methane was detected. They also found evidence that the aromatic ring was cleaved and that the products were fermentable to methane and CO_2.

Healy and Young (1979) demonstrated that eleven simple aromatic lignin derivatives including phenol and catechol were biodegradable to methane and CO_2 under strict anaerobic conditions. They found that the microbes were able to metabolize several different aromatic structures, as long as the aromatic structures were similar; for instance, a culture that had been previously acclimated to vanillic acid could reduce the normal 4-week lag or acclimation period required for catechol decomposition to two weeks.

Suidan et al. (1980) reaffirmed that catechol was anaerobically degradable using an activated carbon upflow filter. An influent catechol concentration of 980 mg/L was adsorbed and anaerobically degraded to methane resulting in an effluent concentration of less than 1 mg/L.

Khan et al. (1981) also used activated carbon columns in their anaerobic phenol experiments. They found high phenol and COD removal efficiencies using influent phenol concentrations of 200, 400, and 1000 mg/L.

Fedorak (1984) has done extensive anaerobic work with phenolics. He found that several non-fermentable alkyl phenolics (such as o- and m-cresol, and isomers of dimethyl phenol) were not particularly inhibitory to the anaerobic process.

Using an anaerobic serum bottle technique (Miller and Wolin 1974), Fedorak found that phenol, at concentrations up to 500 mg/L, was fermentable to methane. Between 800 and 1200 mg/L phenol, methane production was neither enhanced

nor inhibited, while phenol levels greater than 2000 mg/L caused inhibition.

Acclimation of the anaerobic cultures was found to be concentration dependent, with higher concentrations requiring longer acclimation times. Concentrations up to a threshold level of 300 mg/L phenol required 15 days acclimation time, while 400 and 500 mg/L phenol concentrations required 18 and 26 days respectively. Acclimation to p-cresol exhibited similar trends except acclimation times were longer.

Further experiments showed that phenol degrading acid formers were more susceptible to inhibition by phenol than methanogens or non-phenol degrading acid formers. This is a significant finding because it is commonly found that methane bacteria are more sensitive than acid forming bacteria.

3.5 THE ANAEROBIC TREATMENT OF SIMILAR WASTEWATERS

The criteria for examining research dealing with a particular wastewater are partly based on its resemblance to the chemical make-up of refinery sour water. However, other relevant points to investigate from these anaerobic research endeavours are:

- The techniques and materials used
- The type and degree of pretreatment given the wastewater
- The methods employed to prevent digester toxicity
- The degree and rate of organics reduction

There is no specific literature available on the anaerobic treatment of refinery sour waters. American Petroleum Institute (1969) reported one instance of an anaerobic lagoon being used to treat refinery wastewaters, but there were odour problems because of the presence of sulphur compounds.

Research on other wastewaters with similar compositions, such as shale-oil retort wastewater and coal gasification wastewater, is worth investigating. Shale-oil retorting wastewater exhibits rough qualitative similarities to refinery sour water in that both contain significant phenol, ammonia, and sulphide concentrations, and both contain many heavy metals. However, there is some evidence of their differences. Wingender et al. (1981) compared a shale-oil retort wastewater and a conventional crude sour wastewater to determine if an existing crude-oil refinery wastewater treatment process would be adequate for retorting wastewater. The shale-oil and conventional crude were alternately processed and the effluents from an isocracking unit were analyzed for organic constituents.

Wingender et al. (1981) found that the predominant types of organic compounds identified in the shale-oil retort water were pyridines and anilines. By contrast, straight-chain alkanes were the predominant organics formed in the sour condensate from conventional crude.

The authors suggested that because of the predominance of organic nitrogen compounds in shale-oil condensate

relative to crude oil condensate, modifications in the existing sour water treatment train might be necessary. The modifications suggested were limited to pretreatment, for example, the addition of caustic to raise the pH in the stripping system.

Differences between the two wastewaters are to be expected because of the different crudes and different extraction techniques. However, the magnitude of difference between various sour waters in general is so large that it is impossible to conclude categorically that shale-oil retort wastewater is very different from sour water. The literature (particularily Raphaelian and Harrison 1978) indicates sufficient qualitative similarities between the two wastewaters to warrant further investigation.

All studies undertaken on the anaerobic treatment of shale-oil retort water indicated the necessity for pretreating the wastewaters before anaerobic treatment. This was due in part to the high levels of ammonia in the wastewater. Effective pretreatment was attained by a laboratory-scale stripping operation (Mercer et al. 1982, Ossio et al. 1978, and Monsanto Research Corporation 1981). Pretreatment to remove arsenic was practiced sometimes.

Mercer et al. (1982) conducted several tests on shale-oil retort wastewater with two 3.5 liter completely mixed anaerobic reactors. They found that activated carbon as a digester supplement at a 2000 mg/L concentration was essential for consistent maintenance of gas production. They

indicated that activated carbon successfully reduced the toxic effect of inhibitory substances, even heavy metals. When digester failure occurred it was suspected to be due to the presence of ammonia, sulphate, thiosulphate, arsenic, and possibly heavy metals, depending upon the sample source, the concentration used, and the degree of pretreatment the wastewater received.

Additional work by Ossio et al. (1978) on shale-oil retort water demonstrated its anaerobic degradability in lab-scale batch reactors. Pretreatment to remove ammonia and sulphide was necessary. BOD_5 and COD removals of 76 to 80% were obtained. An indication was given that the methane bacteria were nutrient limited.

There have also been several studies aimed at assessing the anaerobic degradability of coal-gasification wastewaters. These wastewaters tend to have a much higher but similar organics concentration compared to refinery wastewaters.

According to Singer et al. (1978) coal gasification wastewater is composed of six major organic groups: monohydric phenolics, dihydric phenolics, polycyclic hydroxyl compounds, monocyclic n-aromatics, polycyclic n-aromatics, and aliphatic acids. Of these, phenol, methylphenol and C_2-phenolics constitute 60 to 80% of the organic content in these wastewaters. Cyanide, thiocyanate, and ammonia are some inorganic compounds of concern.

Chmielowski and Kushnik (1966) initiated research on anaerobic treatment of coal gasification wastewaters. They used tightly sealed, 3-liter glass bottles as batch reactors. One liter of fermentative mass was added to each reactor, and incubated at 32°C.

Three different wastewaters were evaluated for organics degradation; one (which will not be discussed as it is not relevent) was a wastewater derived from a phenol synthesis plant. The other two were from a plant that chemically converted coal to gas: one was from the spent liquor from the gas works (gas generator waters), and the other was the aqueous effluent from a coke oven.

These wastewaters were composed of a complex mixture of phenolics, aromatic bases, organic acids, and non-organic compounds. Cyanide and ammonia were the only non-organic constituents identified. The gas generator wastewaters contained total phenol levels of 5645 to 7750 mg/L while total phenolics in the wastewaters derived from the coking of coal ranged from 5320 to 7270 mg/L. Ammonia was removed prior to use and the pH was adjusted to 7.0 using sulphuric acid.

The coal gasification wastewaters were fermented (900 mg/L total phenolics concentration) only when the inoculum was previously acclimated to pure phenol. Different fermentative responses for the two wastewaters resulted and was attributed to different proportions of mono- and polyhydric phenolics. However, the systems became inhibited

when the inoculum was not previously acclimated to pure phenol, in spite of a gradual introduction of the wastewaters. Chmielowski and Kushnik (1966) speculated that this was due to an inhibitory effect from numerous non-phenolic or non-fermentable phenolics. If the system was previously acclimated to pure phenol these otherwise inhibitory compounds did not exert their toxic effects.

Suidan et al. (1983), after several previous studies using synthetically prepared wastewaters, successfully achieved anaerobic degradation of a 10% dilution of coal-gasification wastewater from a pilot plant. They used an upflow granular activated carbon filter.

Relatively high levels of cyanide (25 mg/L) and ammonia (1040 mg/L) apparently did not hinder operations. No cyanide or thiocyanate removals were achieved in the filter. Excellent removal efficiencies of DOC and TOC were achieved. There was evidence for bioregeneration of the activated carbon.

3.6 POSSIBLE TOXICANTS IN SOUR WATER

Most industrial wastes are toxic at some concentration or dilution. Inhibition of a microbial system may result from a general effect on the environment as a whole, such as pH, or from effects on specific cells or enzymes within the process.

Enzyme inhibition may result from competition between inhibitor and substrate for attachment sites on the enzyme,

an attraction of the inhibitor for a part of the enzyme, or destruction of an essential functional group of the enzyme (Hovious et al. 1973).

Chemical inhibition on a cellular constituent may be the result of a reaction with an essential group of a protein or other molecule, or adsorption at the cell surface. Phenolics are inhibitory due to interference with cellular permeability, while mercury will interfere by a mercury-thiol bonding with an essential protein group. Hovious et al. (1973) noted that:

> "The relative effect of any inhibitory material on a microbial process may be expected to depend on the properties of microbial growth. As an example, rapidly growing cells would be expected to be more susceptible to inhibitory materials than slower growing cells since a higher uptake and conversion of substrate is in progress. Also, microorganisms which grow in clusters or have slime layers would be expected to be less sensitive than dispersed organisms as less opportunity is available for inhibitor-organism contact."

Failure of biological wastewater treatment systems in refineries is often attributed to high phenol surges, or high sulphide or ammonia concentrations. High salt concentrations have been found to be responsible in at least one case (Volesky et al. 1983).

The most obvious potential toxicants (sulphide and ammonia) are those which standard pretreatment systems are designed to remove. Sulphide becomes toxic anaerobically when concentrations exceed 200 mg/L (Lawrence et al. 1964). Parkin and Miller (1982) determined that the maximum tolerable concentrations of ammonia nitrogen to anaerobic

serum bottle cultures at 35°C, pH 6.6 to 7.2 and 15, 25, and 25 days solids retention time were 6000, 7800, and 7600 mg NH_3/L respectively.

Cyanides are another potential source of toxicity. Prather and Berkemeyer (1975) investigated levels of cyanide produced by various processes within a refinery and the reductions afforded by steam stripping pretreatment. They found that anywhere from 23 to 144 mg/L of cyanide were produced from the catayltic cracker and that this source contributed 98% of the cyanide to the total refinery wastewater stream. Excellent cyanide removals were achieved, however, in the stripping unit, which was specifically designed for sulphide removal.

A study by Yang et al. (1980) indicated cyanide inhibition at levels of less than 1 mg/L in unacclimated anaerobic filters. With acclimation the microbes could tolerate 20 to 40 mg/L of cyanide.

Heavy metals also have an attraction for cyanides and sulphides; metal-sulphide precipitates are formed depending on the solubility constants, but cyanide-metal complexes are usually very soluble. The toxicity of the metal-cyanide complex formed depends on the particular metal. Ferrous-cyanide complexes are very stable and therefore not very toxic (Benefield et al. 1982).

Several heavy metals may be found in refinery effluents. The major sources of these metals are the crude oil and anti-corrosion products.

Ions such as chloride and sodium may be found in inhibitory concentrations particularily if crude desalter water forms a major portion of the wastewater.

Anaerobic treatment of wastewaters from petrochemical and coal-gasification industries have been complicated because of the inhibitory effects of various organic constituents. However, little is known about organics other than phenolics in sour water. The inhibitory concentrations of some phenolics was previously discussed.

3.7 PRETREATMENT ALTERNATIVES FOR ANAEROBIC TREATMENT

It is possible to pretreat any wastewater to the point that it is biologically treatable, however, economics will usually limit the degree of steam stripping pretreatment to the point that it is just amenable to biological treatment.

Pretreatment can be useful in helping to determine which wastewater components might be toxic. For instance, if the addition of activated carbon enabled an otherwise inhibitory wastewater to become immediately non-inhibitory, then one would strongly suspect that the inhibition was being caused by organic toxicants.

Three separate pretreatment alternatives are now discussed.

3.7.1 Activated Carbon Pretreatment

The ability of activated carbon to adsorb organics and to some degree inorganics, such as heavy metals, is well

known (Suidan et al. 1979).

Its use as an additive for activated sludge treatment or as a complete secondary or tertiary treatment for refinery sour waters, is becoming more common. Activated carbon is especially effective in removing non-biodegradable refractory organics. Suidan et al. (1979) reviewed activated carbon use for phenolic wastewater treatment in general. They noted that the ability of activated carbon to adsorb phenol relative to many other organics makes it an attractive treatment system for many of these wastewaters.

Monsanto Research Corporation (1981) found activated carbon effective for removing heavy metals in addition to removing organics from the shale-oil retort water they were studying as a candidate for anaerobic treatment.

Mercer et al. (1982) used activated carbon as a digester additive to assess the treatability of three shale-oil retort wastewaters. The dosages applied were 1500 and 2000 mg/L activated carbon. The COD of the various wastewaters ranged from about 4000 to 8000 mg/L. They concluded that activated carbon was essential in reducing toxicity. Suspected toxicants, when inhibition occurred, were ammonia, sulphur as sulphate and thiocyanate, arsenic, and possibly heavy metals.

3.7.2 Stripping

The conventional pretreatment given refinery sour water prior to biological treatment is steam stripping or

oxidation at high temperatures and pressures. Most pretreatment systems are designed to remove sulphide and secondarily, ammonia.

Sulphide removal as H_2S at neutral pH is fast and effective, even at room temperature. However, ammonia removal is negligible even at high temperatures, unless the pH is raised. Ammonia removal at room temperature is negligible regardless of the pH. Kerr (1977) attributed this to the complexing action of weak organic acids and both weak and strong organic sulphur acids with ammonia. The only practical way of removing ammonia is to suitably adjust the pH and raise the sample temperature.

3.7.3 Metal scavenging

When metals toxicity is suspected, chemical treatment is most effective.

Mercer et al. (1982) treated steam stripped shale-oil retort water with 100 mg/L Fe^{3+} (as $FeCl_3$) at pH 6.0, or 100 mg/L Fe^{3+} with 32 mg/L Mn^{2+} at pH 7.0 to reduce arsenic from 4.1 mg/L to less than 1 mg/L. Pretreatment with lime alone prior to anaerobic treatment reduced arsenic to acceptable levels.

Copper was reduced in the same study by a factor of 2.6 by $Fe(OH)_3$-MnO_2 scavenging, but other metals, including Co, Sb, Se, Mo, and V, were reduced by a factor of less than two.

4. RESEARCH OBJECTIVES

As long as appropriate pretreatment is performed the phenol in refinery sour water should be anaerobically dregradable. Therefore, determining an appropriate pretreatment system could be considered the primary objective of this research.

Another objective was to try to determine the toxic components remaining after pretreatment. The approach consisted of separating the organic and inorganic components in the sample and incubating the anaerobic cultures using each of these phases to determine if one or both were toxic.

A third objective was to determine which bacterial component was inhibited. This involved spiking the culture with a methanogenic specific substrate, acetate.

The final objective was to determine whether the bacteria were in a constant state of adaptation to the main sample organic, phenol. This could be determined by spiking existing cultures with fresh sample or pure phenol after they passed their active degradation phase, i.e., after the methane response curves have leveled off.

5. GENERAL EXPERIMENTAL METHODS

5.1 THE MODIFIED HUNGATE BATCH ANAEROBIC SERUM BOTTLE TECHNIQUE

The batch anaerobic serum bottle technique used in this research is a modification of the technique first developed and described by Hungate (1950). Since then, several modifications have taken place. The technique used in this research most closely resembles the serum bottle modification developed by Miller and Wolin (1974).

The method used 78 mL serum bottles as batch anaerobic reactors. The following was added to each bottle: 4 mL medium, 10 mL sample and distilled dilution water, and 6 mL inoculum; for a total culture volume of 20 mL. A redox indicator, resazurin, was used to indicate whether the culture was in a reduced or essentially anaerobic state. Care was taken to exclude air from the entire operation and to equilibrate the added solutions with a 30% CO_2-70% N_2 gas mixture. The pH was carefully maintained at 7.0; the bottles were sealed with butyl rubber stoppers and crimped aluminum caps. Three bottles of each dilution or control were prepared. The cultures were then incubated at 37°C for periods ranging up to 100 days. The extent of degradation was monitored by sampling the headspace gas in the serum bottles for methane. The mean methane content for each dilution was then compared to a control using the statistical method of Dunnett (1955).

5.1.1 Procedure

5.1.1.1 Gas Mixture

An oxygen-free gas mixture was used to flush air from the serum bottles and to equilibrate the added solutions.

The gas mixture used consisted of 70% N_2 and 30% CO_2. To ensure oxygen exclusion the gas was first passed through a column of hot reduced copper wire. A separate low quality gas was periodically used to regenerate or reduce the copper column. The gas was delivered via butyl rubber tubing with stainless steel 0.16 cm O.D. tubing end sections. With this arrangement the tube could be inserted into the culture bottles for gasing and then the rubber stoppers could be inserted to form a seal with the tube still in place and allow a positive pressure to build up in the bottle before the tube was slipped out. This would prevent air contamination.

5.1.1.2 Culture Dilution Water

The required culture dilution water (up to 10 mL in the case of the control or none in the case of a 50% sample dilution) was added by pipet to previously gas flushed serum bottles. Once the water was added it was bubbled through with the gas for 30 seconds to strip out oxygen. Thereafter the serum bottle headspace was flushed with the gas mixture.

5.1.1.3 Medium Preparation and Addition

The stock solutions used to prepare the medium are shown in Table 2, and the medium composition is presented in Table 3.

The medium (without the sludge and $NaHCO_3$) was brought to a boil on a hot plate; boiling was continued for 2 minutes, and then 20 mL of filtered anaerobic sludge (using a Whatman #1 filter) was added. The solution was boiled for an additional minute and the flask containing the medium was then placed on ice for rapid cooling. When the solution reached room temperature, 0.34 g of $NaHCO_3$ was added. The solution was then bubbled with the gas mixture until the pH reached 7.0.

Four mL of this medium was then added, using an automatic pipet, to the continuously gas flushed serum bottle containing the previously added culture dilution water. The transfer of the medium using an automatic pipet was carefully controlled to prevent the inclusion of air. This was achieved by repeatedly pumping the pipet end in the gased headspace of the medium flask.

Table 2. Stock solutions used in growth medium.

SOLUTION	COMPONENTS	CONCENTRATION IN DISTILLED WATER (g/L)
Mineral I [1]	NaCl	50
	$CaCl_2.2H_2O$	10
	NH_4Cl	50
	$MgCl_2.6H_2O$	10
Mineral II	$(NH_4)_6Mo_7O_{24}.4H_2O$	10
	$ZnSO_4.7H_2O$	0.1
	H_3BO_3	0.3
	$FeCl_2.4H_2O$	1.5
	$CoCl_2.6H_2O$	10
	$MnCl_2.4H_2O$	0.03
	$NiCl_2.6H_2O$	0.03
	$AlK(SO_4)_2.12H_2O$	0.1
Vitamin B	Nicotinic acid	0.1
	Cyanocobalamine	0.1
	Thiamine	0.05
	p-aminobenzoic acid	0.05
	Pyridoxine	0.25
	Pantothenic acid	0.025
Phosphate	KH_2PO_4	50
Resazurin		0.1
2-methyl-n-butyric acid [2]		102
Sulphide [3]	$Na_2S.9H_2O$	25

[1] Dissolved in 0.01 M HCl rather than distilled water

[2] Adjusted to pH 6.5

[3] Prepared in small aliquots in freshly boiled water just prior to use.

Table 3. Growth Medium Composition.

SOLUTION	VOLUME ADDED (mL)
Distilled water	70
Mineral I	1
Mineral II	0.1
Vitamin B	0.1
Phosphate	1
Resazurin	1
2-methyl-n-butyric acid	0.1

5.1.1.4 Sample Addition

The sample was first equilibrated with the gas mixture and then the pH was adjusted. The sample was transferred to the serum bottle with a standard 10 mL pipet. Once the sample was added, the serum bottle was flushed for an additional 45 seconds before the butyl rubber stopper was inserted. To ensure air exclusion, the rubber stopper was partially inserted with the stainless steel delivery tube still in the bottle. In this way, a positive pressure was built up before the gas delivery tube was slipped out. The aluminum cap was crimped on and the serum bottles placed in an autoclave for 20 minutes at 121°C. This ensured sterilization.

5.1.1.5 Inoculum

The inoculum consisted of fresh, actively fermenting anaerobic digester sludge from the Edmonton Gold Bar Wastewater Treatment Plant.

The sludge was continuously stirred using a magnetic stirring rod to ensure solids consistency. The headspace of the flask containing the sludge was continuously flushed with the gas mixture.

Six milliliter aliquots of inoculum were drawn off using a plastic disposable syringe with an 18G-3.8 cm needle and then injected through the butyl rubber stopper into the serum bottle. The bottles were then incubated at 37°C.

5.2 ANALYTICAL METHODS

5.2.1 Methane

Methane in the headspace of the serum bottles was measured using gas chromatography. The same model gas chromatograph and integrator were used as in the phenol determinations.

A 90 cm, 1 mm I.D. glass column, packed with 1% SP-1240 DA on 100/120 Sulpelcoport (Sulpelco Inc.) was used. Oven and injector ports were operated at room temperature and the detector was operated at 100°C. Combustion and carrier gases were as previously described.

Standards, using 158 mL serum bottles, were prepared prior to every analysis. The standards were prepared by injecting known amounts of purified methane into the stoppered serum bottles. The percent methane in each standard was calculated according to:

$$\%CH_4 = X/(X + V) \qquad (3)$$

Where; V = volume of the serum bottle, and,

X = milliliters of CH_4 injected.

A 0.5 mL syringe was used to inject 0.1 mL of these standards into the gas chromatograph. The resulting integrator peak areas were compared to the percent methane

in the standardized serum bottles using straight line regression, with absissa values being peak areas and ordinate values being the percent methane in the standardized serum bottles. The resulting regression equation was then used to compute the percent methane in the culture headspace.

Because the cultures contained liquid but the standards did not, a correction factor accounting for the vapour concentration had to be used.

At a specific temperature saturated air contains a specific weight of water per unit volume (absolute humidity). This weight was read from standard tables (List, 1949) for the temperature at which the samples were being analyzed.

A correction factor was calculated according to the following equation:

$$CF = 1 - m(a/b) \qquad (4)$$

Where: CF = correction factor, expressed as a decimal fraction

a = grams of H_2O/L at specified °C

b = 18 g H_2O per mole

m = 22.4 liters gas per mole at STP

This correction factor was then applied to the sample

methane values.

The statistical analysis of Dunnett (1955) (computerized program developed by Fedorak 1984) allowed a comparison of the mean of each set (always three serum bottle cultures in a set or control) with the mean of a control. The test established a non-significance range ($P<0.05$) about the control. The mean methane value for a set was then compared to this range to determine if it was significantly different from the control.

5.2.2 Phenol

Phenol concentrations were determined using a gas chromatograph (Hewlett Packard 5730 A) equipped with a flame ionization detector (FID). A 1 mm I.D., 200 cm glass column, packed with 10% FFAP on 80/100 chromosorb W AW (Supelco Inc.) was used. The gases used were: N_2 carrier gas at 30 mL/min, H_2 and air at 30 mL/min and 240 mL/min respectively (FID combustion gases). The oven was operated isothermally at 190°C, while the detector and injection ports were operated at 250°C. A Hewlett Packard 3385 A integrator was used for determining the peak areas. Other phenolics besides phenol were present but were not enumerated.

5.2.3 Sulphide

Sulphide determinations were performed according to Standard Methods #427D (APHA 1980), using the iodometric method. The method used did not include a preliminary

precipitation step. A straight titration was judged to be suitable after finding a complete sulphide recovery from sulphide spiked sour water samples.

5.2.4 Ammonia-nitrogen

Ammonia-nitrogen concentrations were analysed according to Standard Methods (APHA 1980). A preliminary distillation step was followed (Standard Methods #417A) and the distillant and indicating boric acid solution were titrated with sulphuric acid (Standard Methods #417D).

5.2.5 Cyanide

Cyanide determinations were performed according to Standard Methods #412B and #412C (APHA 1980). A preliminary distillation step was followed and the cyanide content was determined by the titration method with a standardized silver nitrate solution. Because of the low cyanide values obtained it was decided that sample analysis after pretreatment was not necessary. Very good (i.e., 99%) cyanide recoveries on standards were obtained using this method.

5.2.6 COD

COD determinations were carried out using the Oceanographic International Corporation ampule method (O.I.Corporation)

6. SAMPLE DESCRIPTIONS AND PRETREATMENT METHODS

6.1 SAMPLES

Four separate samples were obtained throughout the course of this research from three local Edmonton refineries. These samples have been designated A, B, C, and D.

Approximately 20 L of sample A were collected from the condensate line of a catalytic cracker. Therefore it was strictly a sour condensate. As a precautionary measure sample A was stripped with CO_2 and N_2 gas upon arrival at the laboratory to reduce the toxic H_2S. This did not involve adjusting the pH. Any reference to N_2 stripping of the samples that follows is not to be confused with this preliminary stripping. The sample was stored in a tightly sealed plastic container at 5°C. The raw sample parameters (after preliminary CO_2 and N_2 stripping) are presented in Table 4.

Sample B (40 L) was handled in the same way as sample A. The contributing processes in the refinery were the catalytic cracker low pressure condensate water and gas treating caustic wash water. The parameters are given in Table 4.

Twenty liters of sample C were obtained from a sampling point which included the following contributing unit processes:

1. The crude second stage receiver

Table 4, Raw and N_2 pretreated sample parameters.

B.P. = before pretreatment
A.P. = after pretreatment
NA = not analysed

PARAMETER	CONDITION	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
pH	B.P.	9.5	9.5	9.1	
	A.P.	6-8	6-8	6-8	6-8
COD mg/L	B.P.	2300	3300	1400	NA
	A.P.	NA	NA	NA	NA
NH_3-N mg/L	B.P.	3680	470	1360	4200
	A.P.	3500	540	1200	3930
S^{2-} mg/L	B.P.	NA	1100	490	5040
	A.P.	50	50	30	50
CN^- mg/L	B.P.	6	2	4	NA
	A.P.	NA	NA	NA	NA
Phenol	B.P.	540	170	140	490
	A.P.	510	160	140	460

Note: Sample A received preliminary pretreatment upon arrival at the laboratory as a precautionary measure. It was further pretreated prior to its addition to the cultures, and the A.P. values refer to this second pretreatment operation.

2. The vacuum unit hotwell
3. The coker overhead receiver
4. The fluid catalytic overhead accumulator
5. The saturate gas plant
6. The hydrotreating unit
7. The syncrude fractionation unit
8. The hydrotreating reaction section
9. The hydrotreating fractionation section
10. The decant slop tank

Four liters of sample D were obtained from the same source as sample A, but six months later.

6.2 PRETREATMENT METHODS

Three methods of non-biological pretreatment were used in this research.

6.2.1 Nitrogen Gas Stripping Pretreatment

The room temperature nitrogen stripping pretreatment alternative involved adjusting the pH of the samples to about 7 and then gas stripping with N_2 (stone diffuser) for a period ranging from 4 to 6 hours. Little ammonia was removed using this technique. One analysis revealed insignificant ammonia removal, even after prolonged stripping. Aside from sulphide removal, most of the other parameters were not affected. Table 4 shows some of the parameters after this form of pretreatment.

6.2.2 Batch Adsorption with Activated Carbon Pretreatment

Activated carbon was used on samples previously N_2 stripped at room temperature.

Calgon 400 activated carbon was applied in dosages ranging to 10 g/L. The activated carbon and sample were placed on a mechanical shaker for seven hours, or as otherwise indicated. The flasks containing the sample and activated carbon were sealed to exclude air and thus prevent oxidation. The activated carbon was separated from the sample before addition to the culture by filtering through a Whatman #1 filter on a Buchner Funnel.

6.2.3 Boiling Pretreatment

Pretreatment by boiling the sample was used to determine whether culture inhibition could be reduced or eliminated.

The samples (sample pH adjusted to 10.0 using 8 N NaOH) were placed in 500 mL Erlenmeyer flasks and brought to a boil on a Corning Hot Plate. The boil was held for approximately 20 minutes. The original volumes, which were reduced by evaporation, were restored using distilled water. The samples were allowed to cool (on the hot plate) for half an hour before the pH was readjusted to 7.0 (using 8 N HCl). The samples were then stripped for one hour using N_2 gas.

Large amounts of precipitate were detected before and after boiling.

7. INDIVIDUAL EXPERIMENTAL PROCEDURES AND RESULTS

The experiments are outlined in sequential order. Each experiment (1 to 6) involved several different sub-experiments. Therefore, to simplify, a coding system was devised.

Each experiment was designated by the main experiment number (1 to 6), a sample letter, and a number relating to the particular sub-experiment. For example, experiment 2B2 denotes experiment 2 using sample B, and involved determining whether the organic or inorganic components in the sample were inhibitory. Table 5 gives the sub-experiment code associated with the experiment and the condition employed. The figure and table numbers corresponding to each sub-experiment are also outlined.

The objectives, sample pretreatment, and procedures are outlined for each experiment. The plots and a brief discussion of the results for all of the experiments are presented in this chapter, and the corresponding tables with the relevant statistical information are provided in Appendix B. Many of the plots have curves deleted but the data are included in the tables (Appendix B). Curves were deleted to prevent cluttering of the figures but the curves that were deleted were selected so as not to exclude significant information or trends. The figure legends include the approximate phenol concentrations (concentrations calculated from values reported in Table 8, Appendix A) that were present in the culture bottles.

Table 5. Sub-experiments conditions.
F = Figure
T = Table

EXPT#	SAMPLE	N_2 STRIPPING[1]	A.C. PRETR.[2]	BOILING PRETR.[3]	ORG./INORG. COMPONENT SEPARATION[4]	ACETATE ENRICHMENT[5]	ADAPTIVE RESPONSE[6]
1	A	1A1 (F1,T9)					
	B	1B1 (F2,T10)					
	C	1C1 (F3,T11)					
2	A	2A1 (F4,T12)	2A3 (F7,T15)		2A2 (F7,T15)	2A4 (F10,T18)	
	B	2B1 (F5,T13)	2B3 (F8,T16)		2B2 (F8,T16)	2B4 (F11,T19)	
	C	2C1 (F6,T14)	2C3 (F9,T17)		2C2 (F8,T17)	2C4 (F12,T20)	
3	A	3A1 (F13,T21)	3A2 (F13,T21)				
	D	3D1 (F14,T22)					
4	A						4A1 (F15)
5	A				5A1 (F16,T23)		
	D			5D2 (F17,T24)	5D1 (F17,T24)		
6	A			6A1 (F18,T25)			
	B			6B1 (F19,T26)			
	C			6C1 (F20,T27)			
	D			6D1 (F21,T28)			

[1] Section 6.2.1
[2] Section 6.2.2
[3] Section 6.2.3
[4] Section 7.2.1
[5] Section 7.2.2
[6] Section 7.4

In the following experiment descriptions, each culture (for each dilution or condition) is represented by three serum bottles, unless otherwise indicated. The figures have smooth curves drawn in for ease of interpretation. The heavier lines represent the controls.

A collective discussion of the results follows in the next chapter.

7.1 Experiment 1

The objective of this experiment was to determine the concentration of N_2 stripped sample (A, B, and C) that would allow effective anaerobic degradation.

Concentrations of 10%, 20%, 30%, 40%, and 50% sample by volume were prepared for incubation. Two controls were also prepared; one was composed of dilution water with no sample and was the reference to which the diluted sample cultures were compared (methane production above or below the control), and the other was a phenol spiked (approximately 300 mg phenol/L) control, which was to indicate when the microorganisms had begun degrading the phenol in the sample cultures.

The results are presented graphically in Figures 1, 2, and 3, and tabulated in Appendix B giving the statistical information (Tables 9, 10, and 11).

7.1.1 Discussion of Results for Experiment 1

The results from the three experiments (Figures 1, 2, and 3) indicate variable methane responses depending on the sample used and the dilution employed.

The cultures containing sample A (Figure 1) displayed the highest methane readings. This is because sample A had the highest phenol concentration (raw sample A concentration: 540 mg phenol/L). The 40% and 50% sample A cultures required considerable time to acclimate to the high

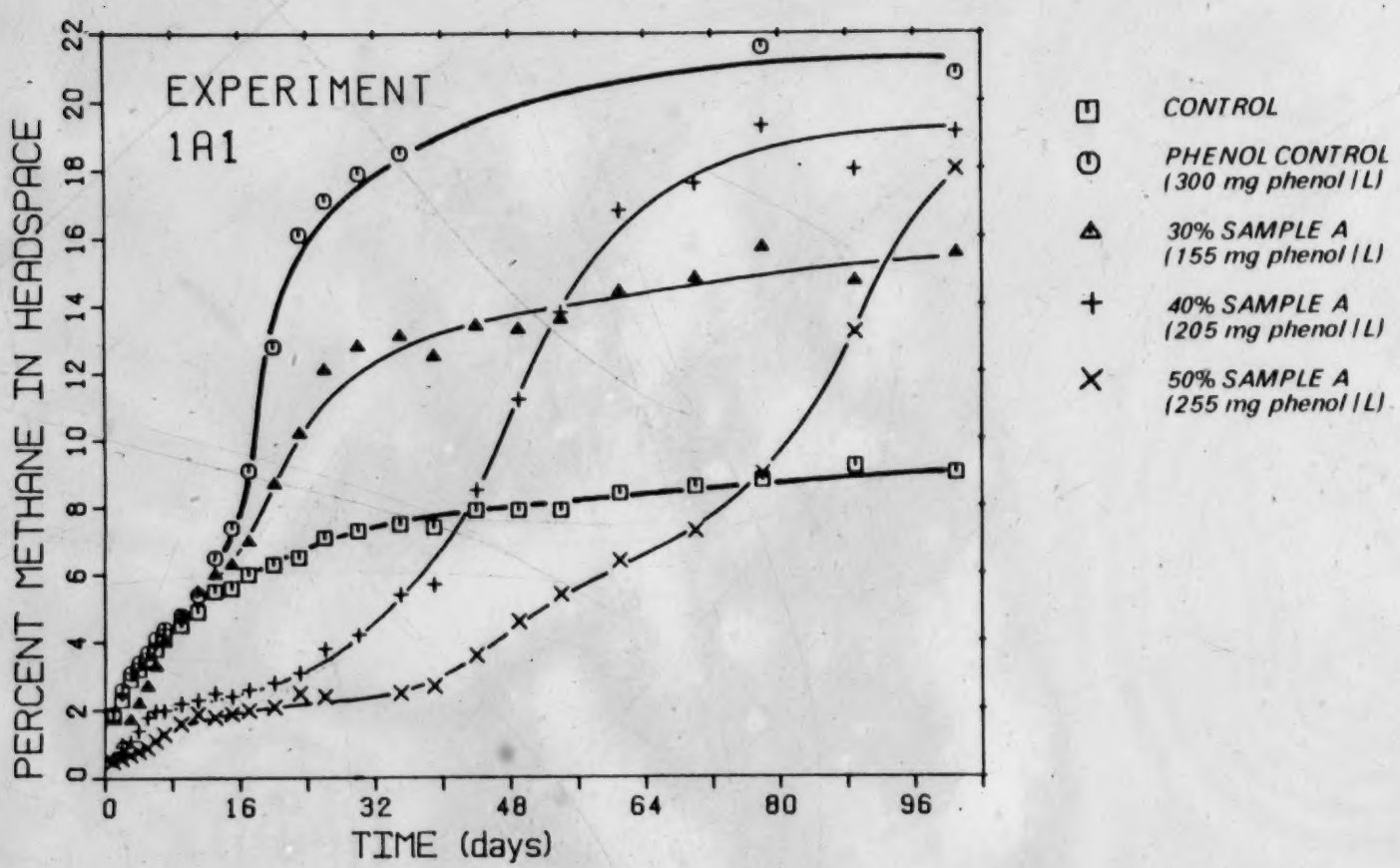

Figure 1. N_2 stripped sample A culture dilutions. See Table 9.

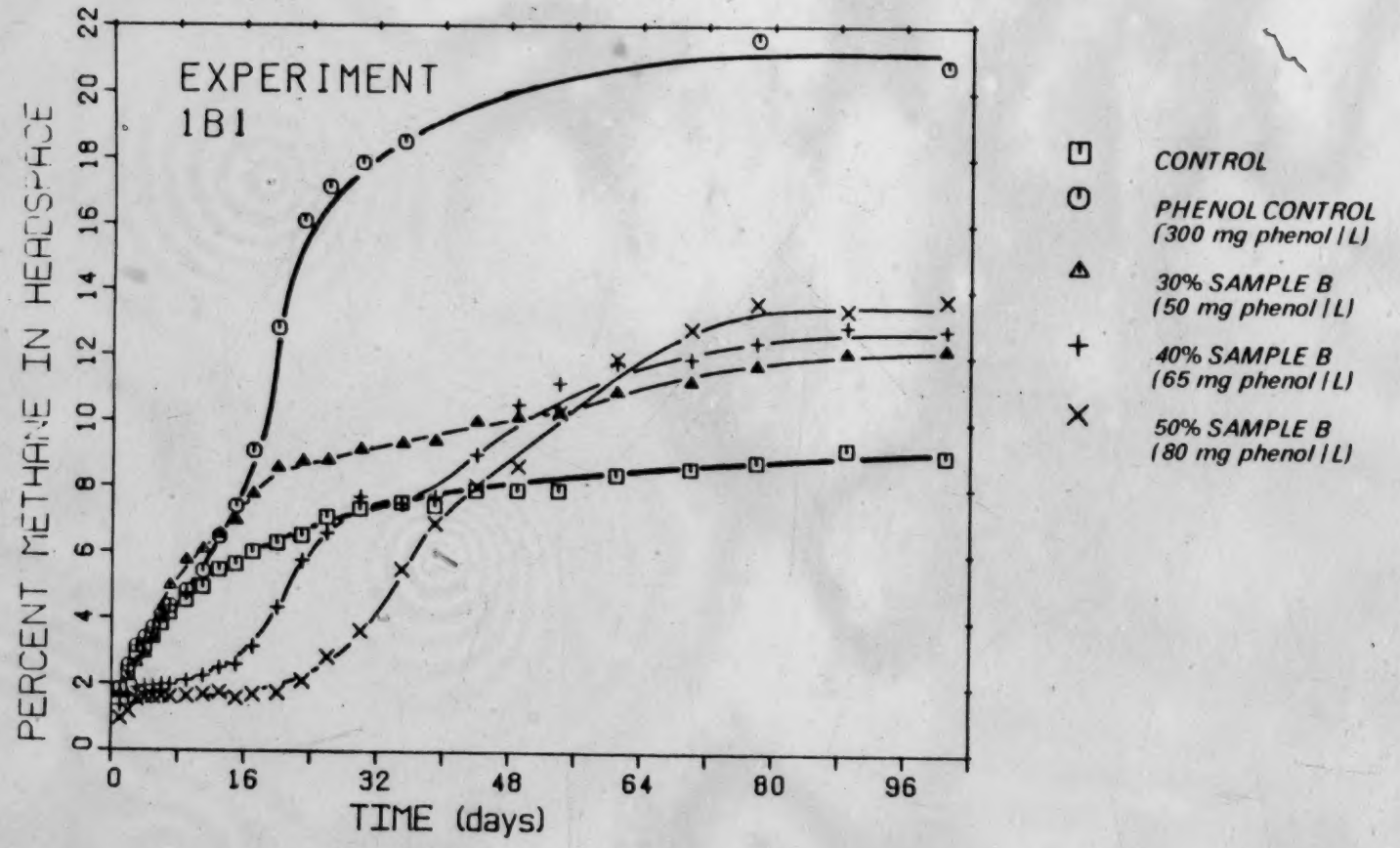

Figure 2. N_2 stripped sample B culture dilutions. See Table 10.

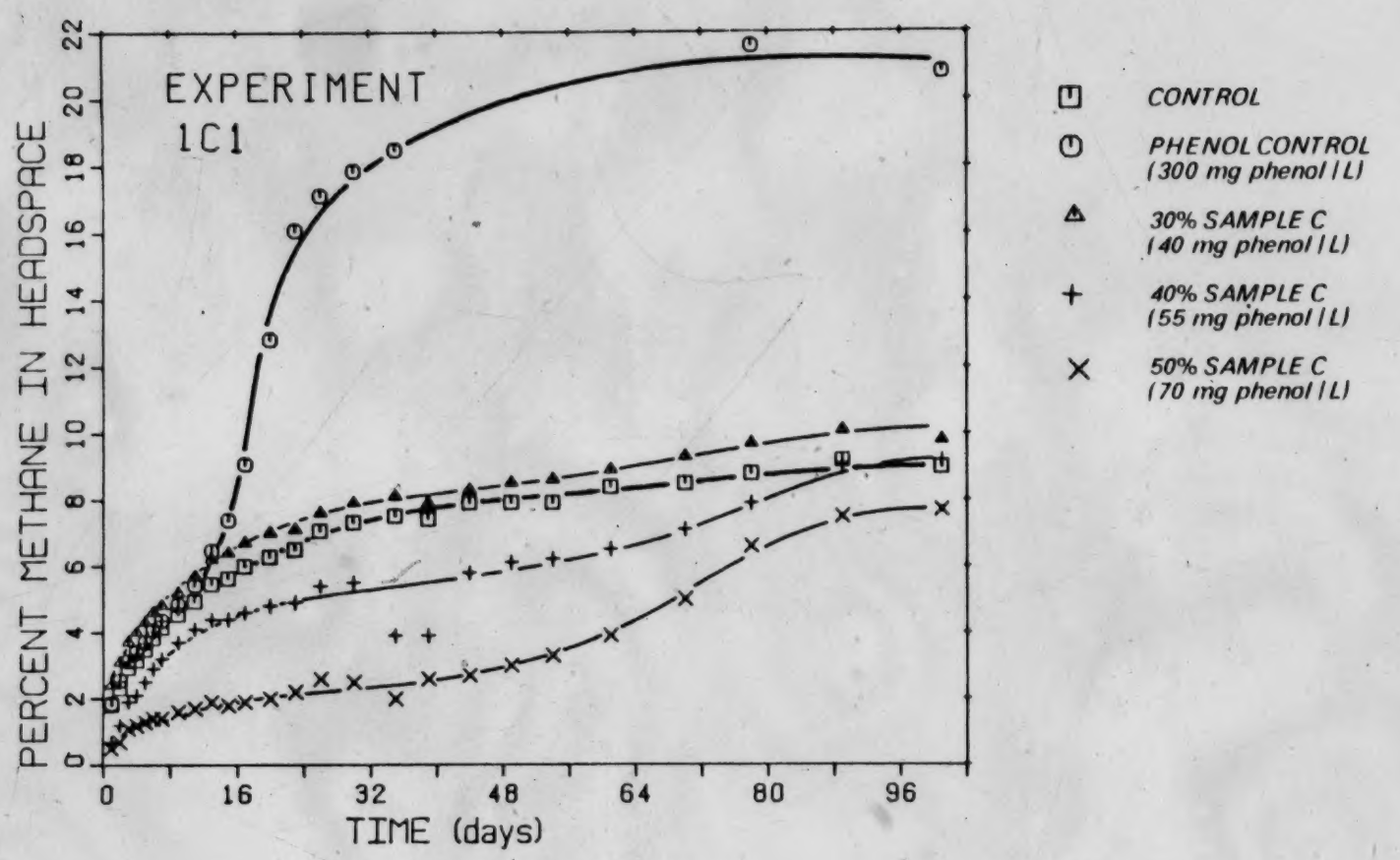

Figure 3. N_2 stripped sample C culture dilutions. See Table 11.

sample concentration (i.e., 39 and 54 days respectively to become non-significantly different from the control). The 10%, 20%, and 30% sample A cultures showed no inhibition.

Similary, the 40% and 50% sample B (Figure 2) cultures were initially inhibited (23 and 35 days respectively), but not for as long as the sample A cultures. The 10%, 20%, and 30% sample B cultures showed no inhibition.

The 40% and 50% sample C cultures (Figure 3) also indicated periods of inhibition (15 and 89 days respectively). None of the sample C cultures displayed enhanced methane production.

Phenol was measured in the 40% sample A culture after the incubation period (101 days), and found to be 13 mg phenol/L (after the culture solution was filtered through a Whatman #1 filter paper). The original phenol concentration in the 40% sample A culture was calculated to be 205 mg phenol/L; therefore, a 94% reduction in phenol occurred over the incubation period.

7.2 Experiment 2

This experiment involved samples A, B, and C. The objectives of this experiment were:

- Determine the repeatability of Experiment 1, which involved N_2 stripping the samples and preparing dilutions.
- Extract the organic constituents from the samples leaving just the inorganic components for culture incubation.
- Determine the effect of batch adsorption with activated carbon on the samples at a 50% sample culture concentration.
- Add acetate (750 mg/L culture concentration), as a strict methanogen substrate, to 50% sample culture dilutions.

7.2.1 Organic Component Extraction

The organic component extraction experiment was designed to provide information about the constituents (organic or inorganic) responsible for inhibition in the 50% concentration samples.

For instance, a reduced methane response of a phenol supplemented 50% inorganic component culture relative to a control would indicate that inorganic constituents are responsible for culture inhibition. There is a chance that organic constituents could be inhibitory also, and this possibility must be investigated. There is also a chance of

an incomplete extraction occurring, leaving some organics, perhaps toxic, with the inorganic components, or vice versa.

The theory behind the technique is to extract relatively non-polar organics into a non-polar organic solvent (diethylether). However, several factors play a role in determining the solubility of a particular organic constituent in an organic solvent. Perhaps the most important is the pH of the aqueous solution. Organic acids increase in solubility in water (decrease solubility in organic solution) with increasing pH, while organic bases (amines) act in an opposite way.

Therefore, an extraction at neutral pH, as was the case in this experiment, cannot be expected to provide perfect component separation. The results must therefore be interpreted with caution.

The procedure used was to adjust the sample pH to 7.0 (previously N_2 stripped) and mix and separate three successive aliquots of diethylether with the sample. The sample volume was 250 mL, and the ether aliquots were 80 mL each. A 500 mL separatory funnel was used.

The resulting 240 mL of ether containing the organics was discarded. The aqueous phase containing only inorganics was then left open to the air overnight to evaporate any remaining ether.

The samples were stripped and equilibrated with the gas mixture prior to use. Phenol was added for a culture substrate (approximately 300 mg phenol/L culture

concentration).

7.2.2 Acetate Addition

Acetate is a strict methanogen substrate so its addition to an inhibited culture (750 mg acetate/L in a 50% sample culture) would provide information as to whether the methanogens were inhibited.

The procedure followed was to prepare a 75 g acetate/L solution, strip and equilibrate it with the gas mixture, and add 0.2 mL of it with a syringe to the culture.

The acetate spiked cultures were statistically compared to the acetate control.

7.2.3 Batch Adsorption With Activated Carbon

Activated carbon was added to N_2 stripped samples in 1, 4, and 10 g/L concentrations. The procedure followed was the same as previously described (Section 6.2.2), using 7 hours contact time for the activated carbon and sample.

The samples were analyzed for phenol but the 10 g/L pretreated samples were found to contain only 10, 0, and 1 mg phenol/L for samples A, B, and C respectively (the samples pretreated with the lower dosages of A.C. had more phenol; Table 8, Appendix A). The cultures containing the pretreated samples (50% concentrations) were spiked with the appropriate mass of phenol to give an approximate final culture concentration of 300 mg phenol/L.

The cultures were statistically compared to the standard control.

7.2.4 Discussion of the Results for Experiment 2

7.2.4.1 Samples N_2 Stripped at Room Temperature

The results for the three samples (Figures 4, 5, and 6) are similar to the previous experiment (Figures 1, 2, and 3).

The curve for the 50% sample A culture (Figure 4) remained inhibited throughout the experiment, in contrast to the Experiment 1 (Figure 1) 50% culture. All of the curves appear to be slightly lower, and where acclimation was necessary, the cultures took slightly longer to respond.

Phenol was measured at the end of the incubation period (91 days) for the 30% and 40% sample A cultures (culture solutions filtered through a Whatman #1 filter paper before analysis), and found to be 52 and 54 mg phenol/L respectively. Assuming original phenol levels of 155 and 205 mg phenol/L, this represents a 66% and 74% phenol reduction respectively.

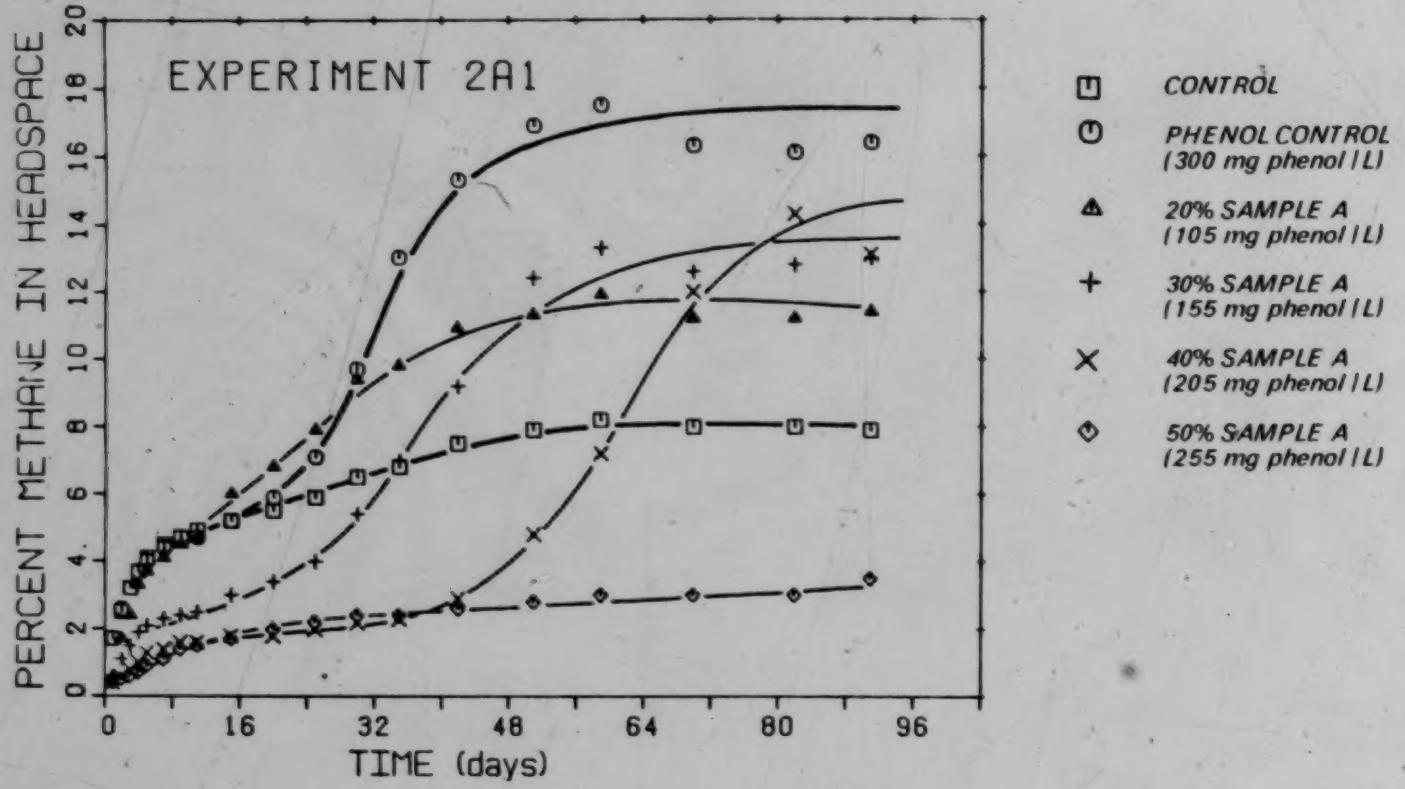

Figure 4. N_2 stripped sample A culture dilutions. See Table 12.

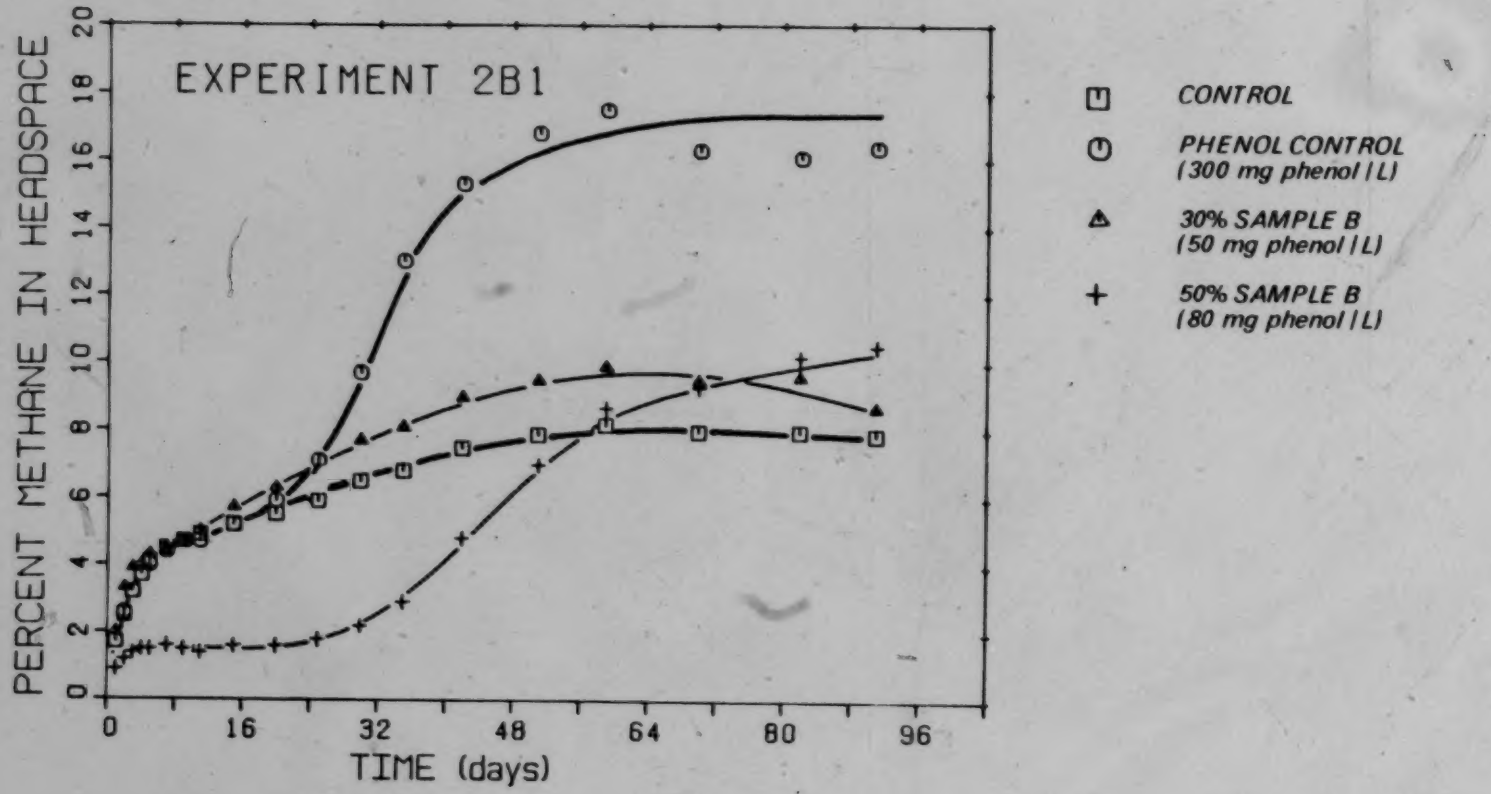

Figure 5. N, stripped sample B culture dilutions. See Table 13.

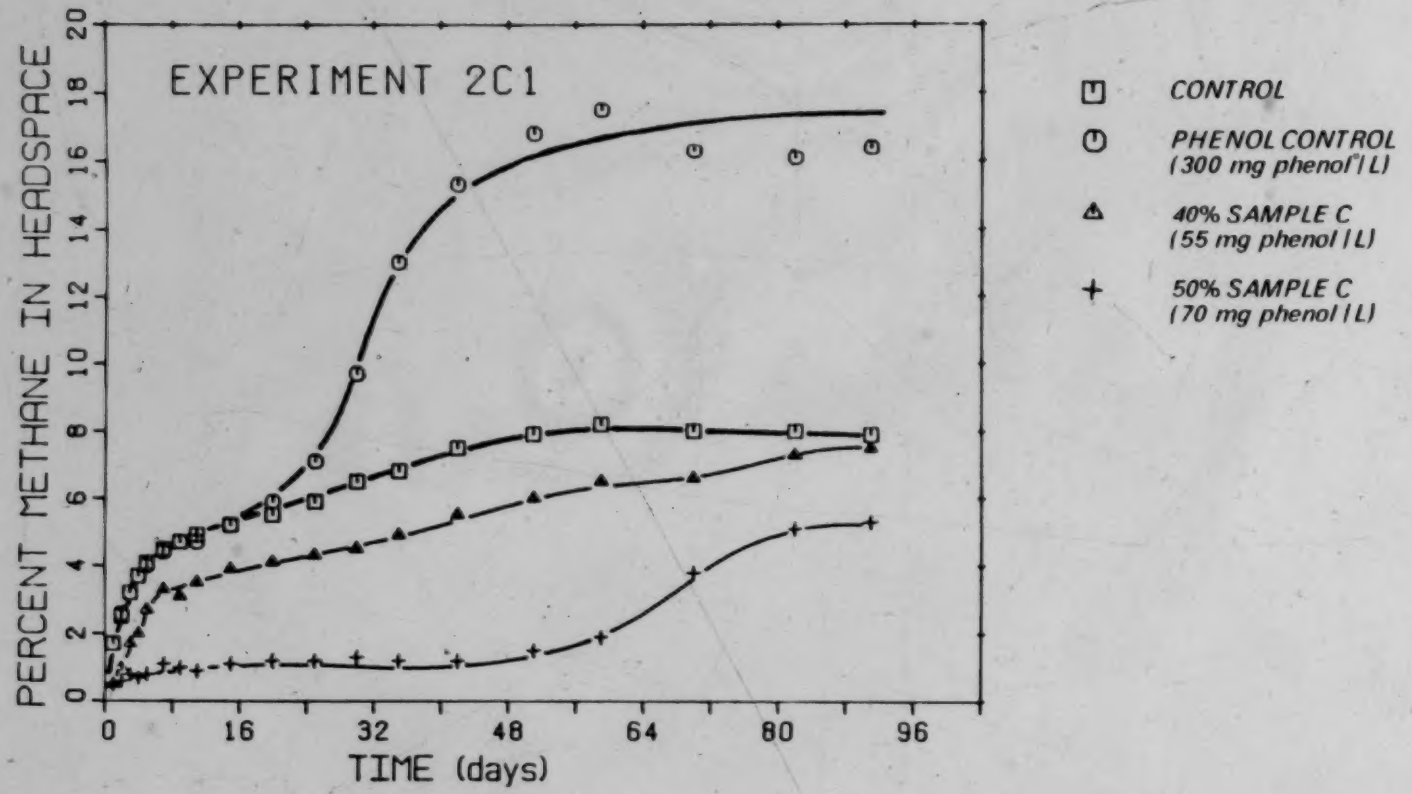

Figure 6. N, stripped sample C culture dilutions. See Table 14.

7.2.4.2 Sample Organics Extraction

The results of these experiments (2A2, 2B2, and 2C2, presented in Figures 7, 8, and 9) are plotted with the curves for the cultures which received sample pretreatment by batch adsorption with activated carbon. The curves for all of the cultures indicate inhibition, which implies that inhibition is being caused by inorganic constituents within the samples.

7.2.4.3 Batch Adsorption with Activated Carbon

The results for the cultures which received sample pretreatment by batch adsorption with activated carbon are presented in figures 7, 8, and 9. A slight methane response improvement for the sample A and B 50% cultures with the 10 A.C./L pretreatment can be noted, although the curves did not indicate statistical methane enhancement relative to the control.

The 1 and 4 g A.C./L sample B pretreated cultures (Tables 15, 16, and 17) indicate about the same response as the 10 g A.C./L cultures. This was not so for the sample A 1 and 4 g A.C./L cultures, where it can be seen that only the 10 g A.C./L dosage had a significant effect.

None of the activated carbon pretreated sample C cultures showed an improvement relative to the unaltered 50% culture.

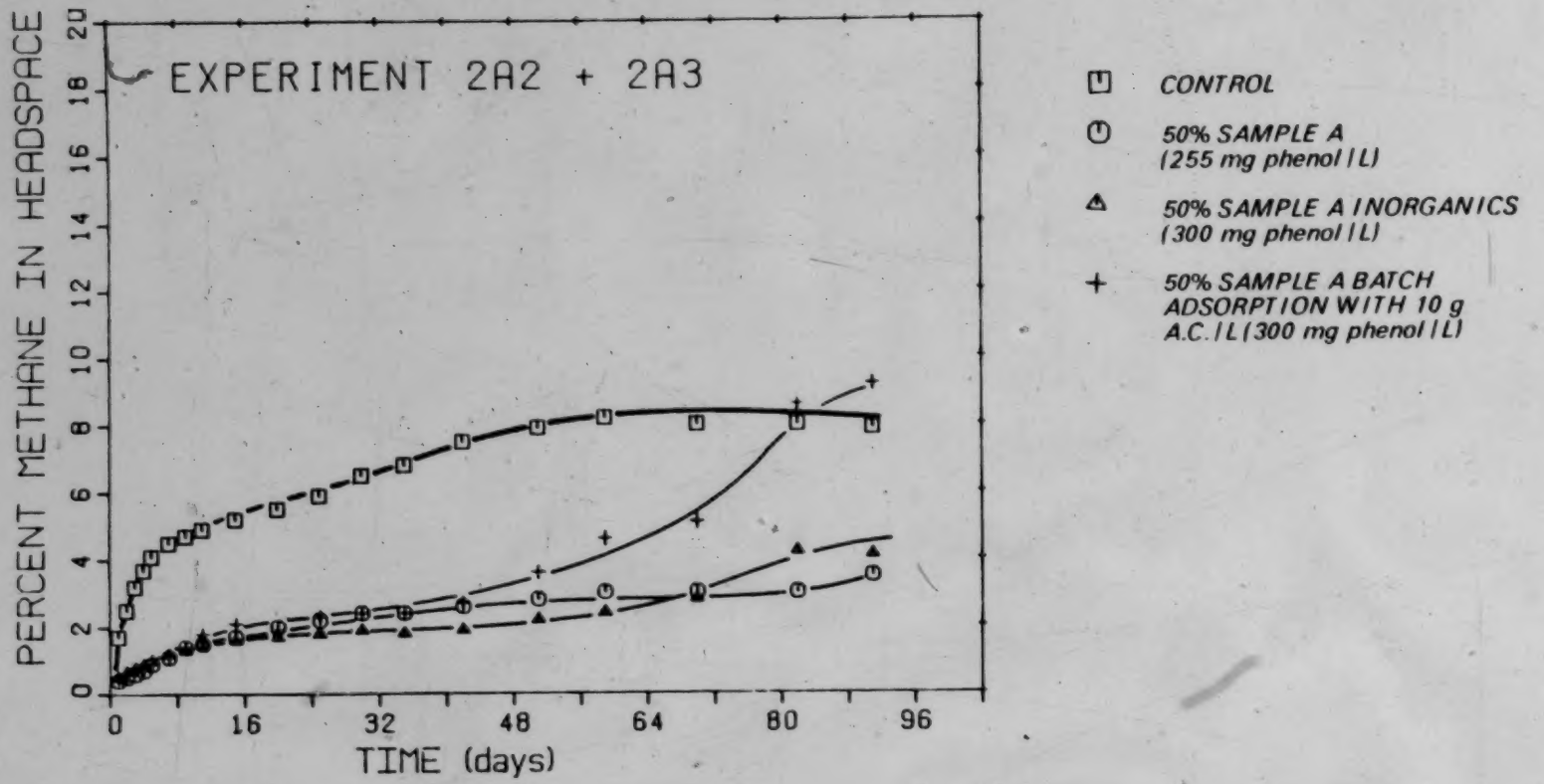

Figure 7. Culture dilutions (50% sample concentration) for: N, stripped sample A, sample A inorganics, and sample A pretreated by batch adsorption with activated carbon (10 g activated carbon/L). See Table 15.

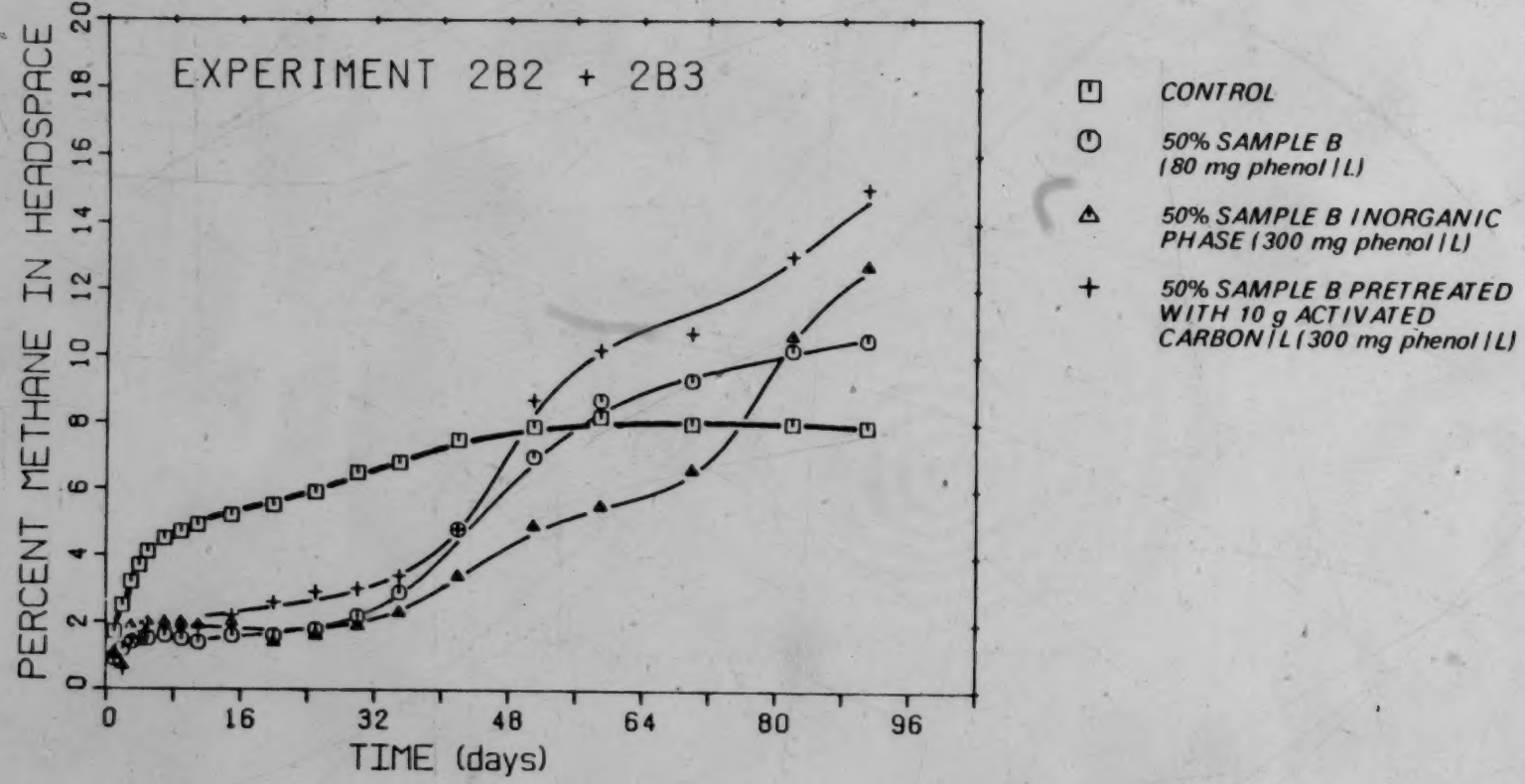

Figure 8. Culture dilutions (50% sample concentration) for: N_2 stripped sample B, sample B inorganics, and sample B pretreated by batch adsorption with activated carbon (10 g activated carbon/L). See Table 16.

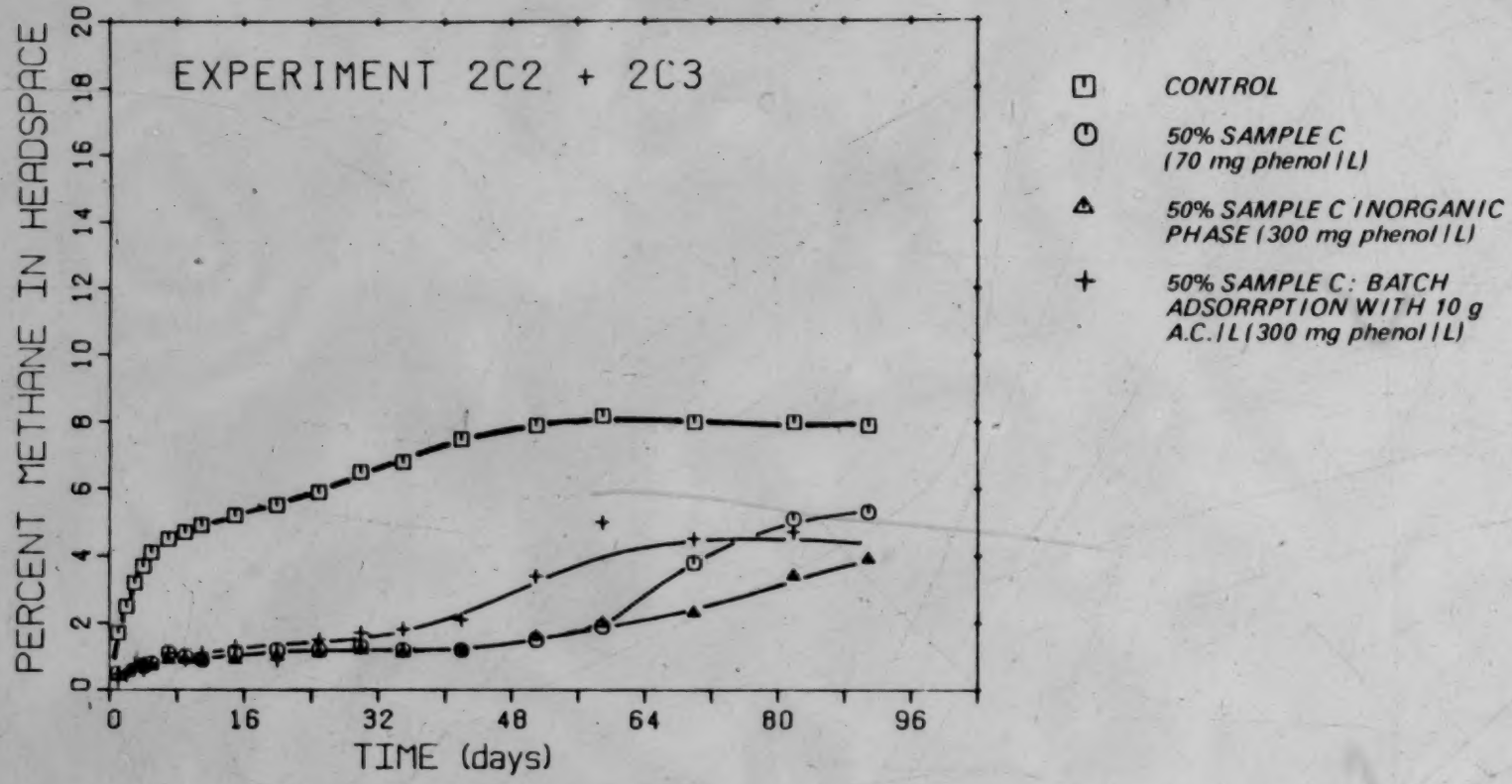

Figure 9. Culture dilutions (50% sample concentration) for: N_2 stripped sample C, sample C inorganics, and sample C pretreated by batch adsorption with activated carbon (10 g activated carbon/L). See Table 17.

7.2.4.4 Acetate Enrichment Experiments

All of the acetate enriched cultures (Figures 10, 11, and 12) showed the acetate cultures responding after a short acclimation period. This is in contrast to the unaltered 50% cultures which, with exception of the sample B culture, were inhibited for the duration of the experiment.

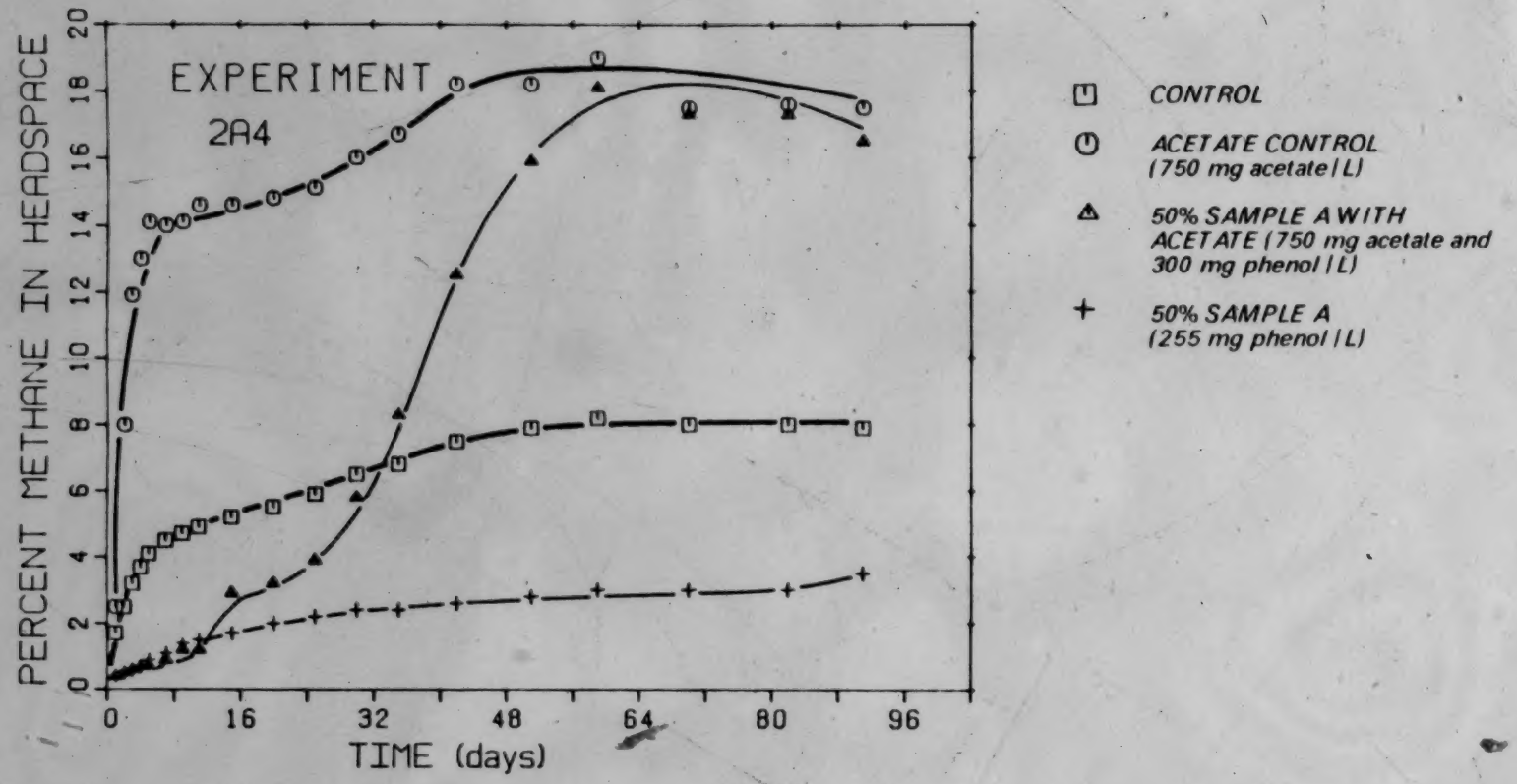

Figure 10. Sample A (50% sample concentration) culture dilution enriched with acetate. See Table 18.

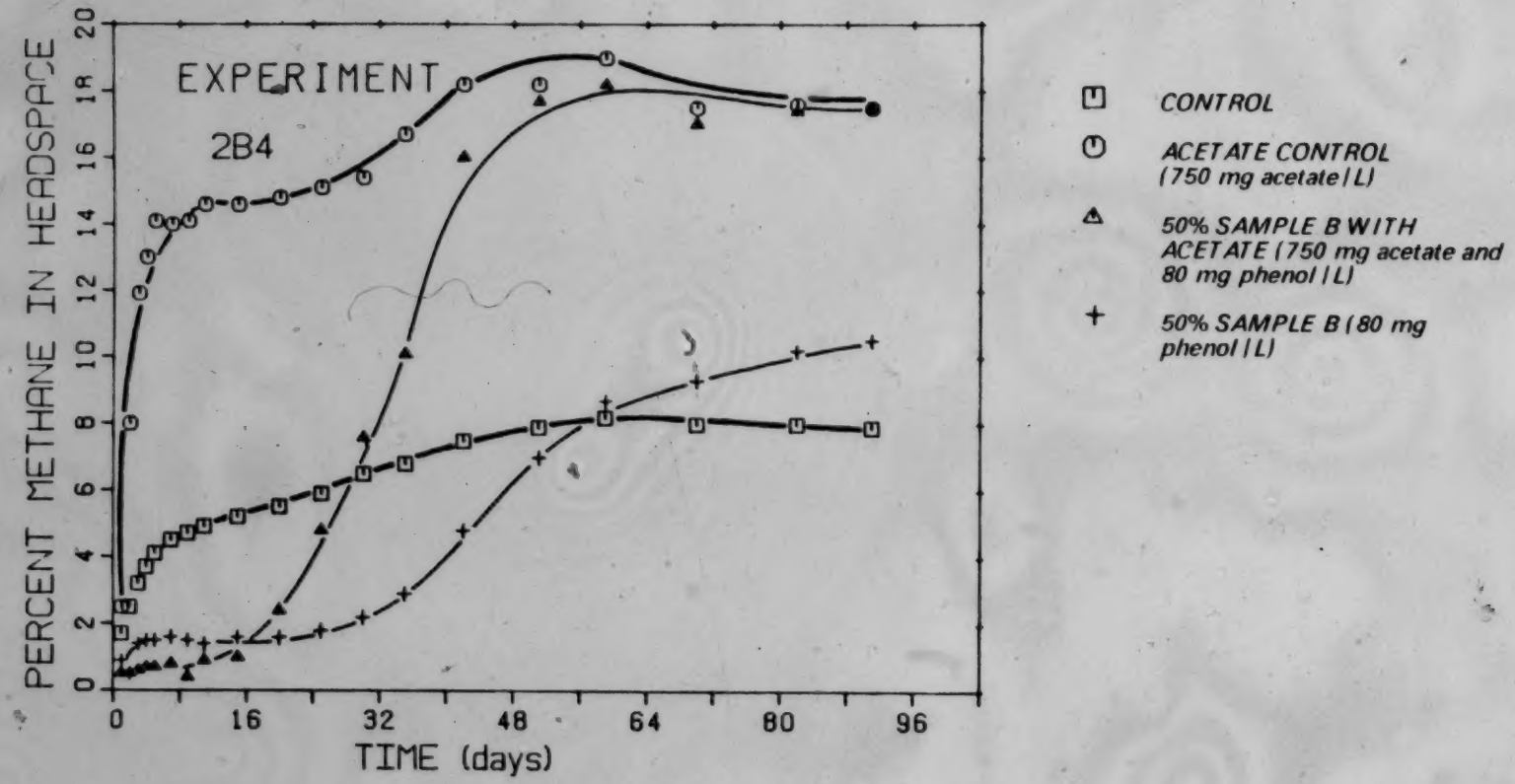

Figure 11. Sample B (50% sample concentration) culture dilution enriched with acetate. See Table 19.

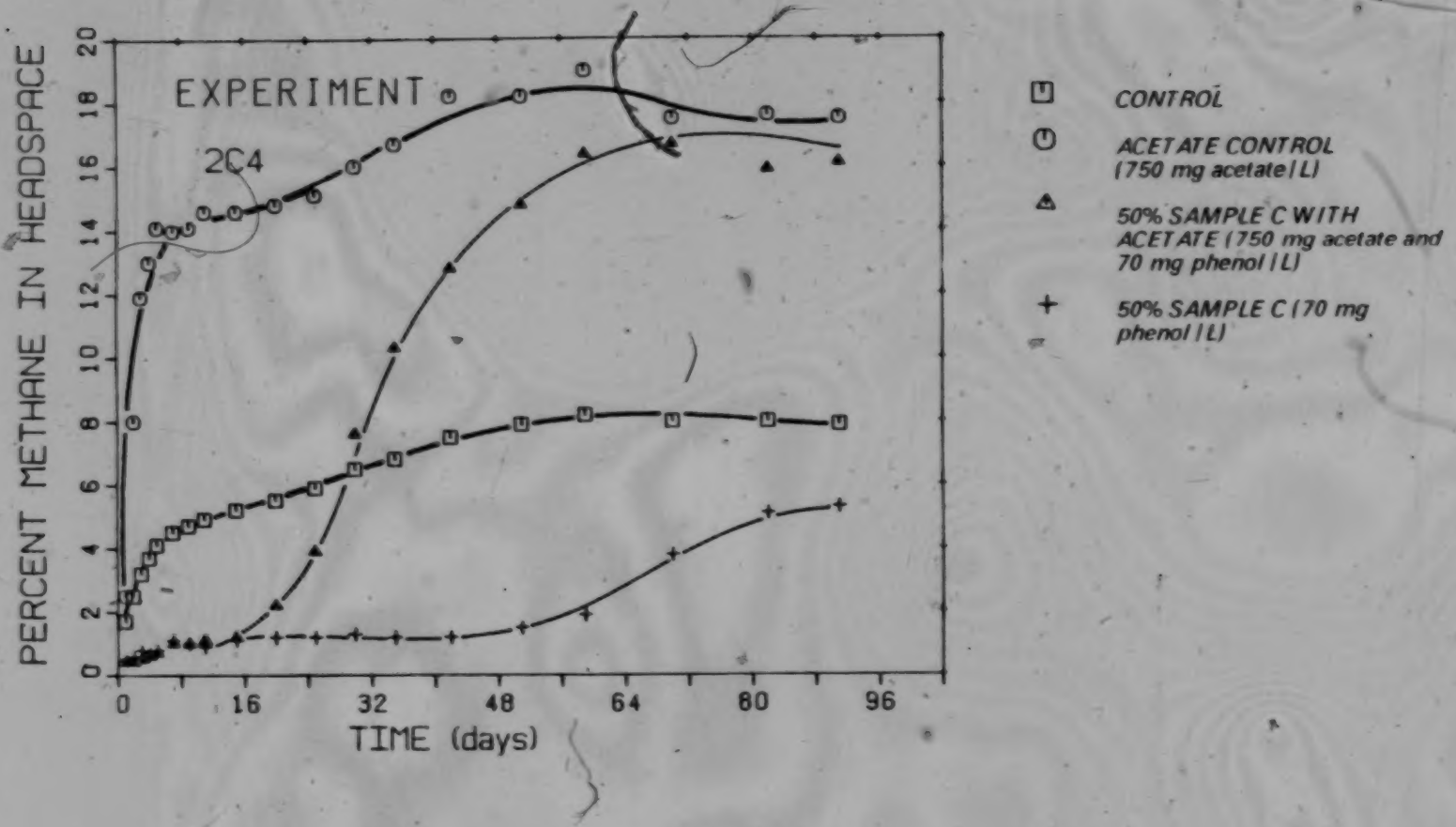

Figure 12. Sample C (50% sample concentration) culture dilution enriched with acetate. See Table 20.

7.3 Experiment 3

This experiment involved samples A and D. The objectives were:

- To again evaluate the response of the cultures to N_2 stripped sample A for 20% to 50% dilutions (Experiment 3A1, presented in Figure 13 and Table 21).
- To re-evaluate the effectiveness of activated carbon adsorption (at 10 g/L) using longer contact times (7 days contact of the activated carbon with the sample A; basic procedure as described in Section 6.2.2) for both 40% and 50% culture concentrations (Experiment 3A2, presented in Figure 13, and Table 21).
- To determine the non-inhibitory sample culture dilutions (10% to 50%) for sample D, after N_2 stripping (Experiment 3D1, presented in Figure 14 and Table 22).

7.3.1 Discussion of the Results for Experiment 3

7.3.1.1 N_2 Stripped Samples

Only the 50% sample A cultures are presented in the plot (Figure 13; the 20% to 40% culture dilutions can be found in Table 21). The methane responses for the curves were similar to the previous two experiments (Figures 1 and 4). The sample D cultures (Figure 14) were all inhibited, at least initially.

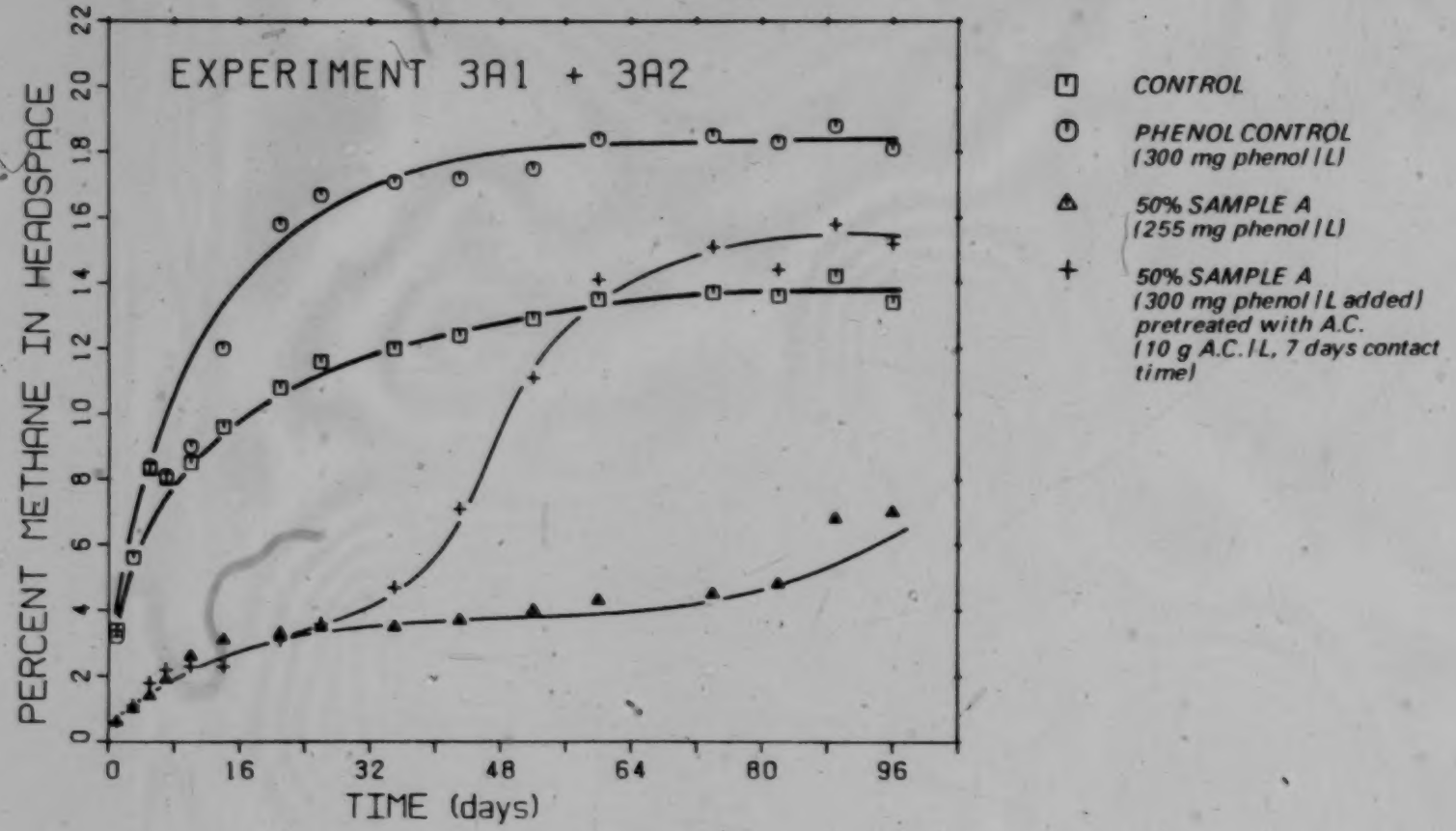

Figure 13. Culture dilutions (50% sample concentrations) for N_2 stripped and batch adsorption with A.C. pretreated sample A. See Table 21.

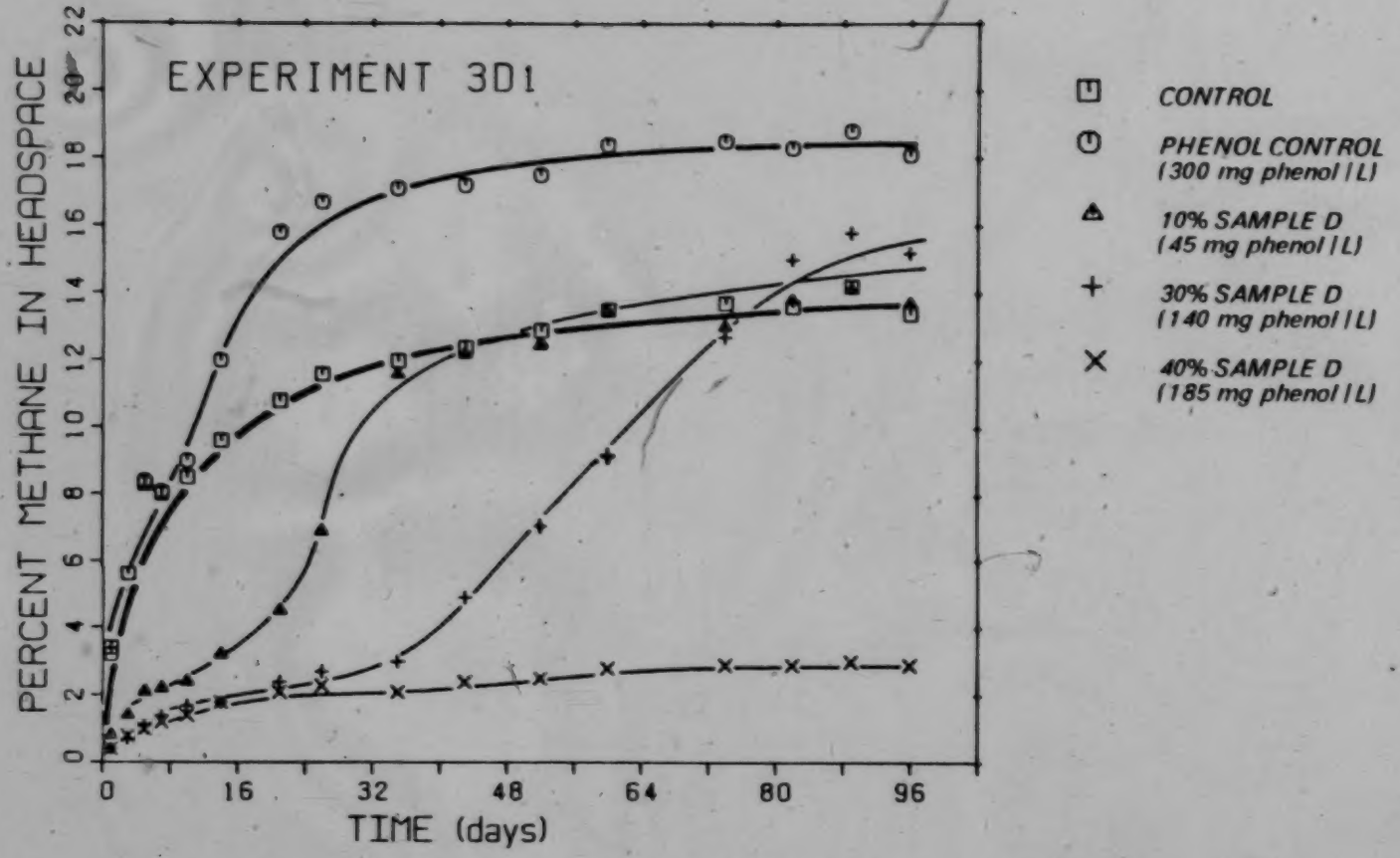

Figure 14. N_2 stripped sample D culture dilutions. See Table 22.

Only the 20% and 30% sample D cultures displayed enhanced methane production relative to the control, but this was only after 96 and 89 days respectively. The 40% and 50% cultures were inhibited throughout the experiment.

7.3.1.2 Batch Adsorption with Activated Carbon

In contrast to the previous batch adsorption experiment (Experiment 2, Figures 7, 8, and 9) this experiment employed only one sample (sample A) but two concentrations (40% and 50%) and a much longer sample-A.C. contact time (7 days as opposed to 7 hours). The longer contact time was beneficial in reducing inhibitory constituents as it allowed the culture microorganisms to acclimate more quickly. The 40% and 50% cultures displayed an immediate methane response relative to the 50% unaltered culture. However, neither the 40% nor the 50% cultures showed an enhanced methane response relative to the control, in spite of there being a 300 mg phenol/L supplemented substrate concentration in the cultures.

7.4 Experiment 4

This experiment involved the use of a 40% sample A culture from experiment 1A1 (see Figure 1), which had reached a methane production plateau (three bottles total).

The objective was to determine whether the microorganisms were in a constant state of adaptation to phenol and the wastewater sample.

The procedure involved withdrawing 8 mL (8 mL in the total culture volume of 20 mL represents 40% of the volume) of supernatant from each bottle. This was achieved by standing the bottles upside down for several days to allow the microorganisms and solids to settle. Then a 10 mL syringe with an 18G-3.8 cm needle was used to withdraw the supernatant.

The bottle headspaces were flushed (while still sealed) with the gas mixture to evacuate any residual methane.

In one bottle, 8 mL of previously boiled and equilibriated distilled water with 1250 mg/L $NaHCO_3$ (pH adjusted to 7.0) was added. This served as the control.

In another bottle, the same procedure was followed but phenol was added to give approximately a 100 mg/L phenol concentration. This serum bottle culture served as the phenol control.

The fresh N_2 stripped sample A was added to the third bottle, but it was first boiled to reduce the oxygen present. $NaHCO_3$ was then added, and the pH was adjusted.

The results are presented in Figure 15, but because there were only three bottles, a statistical evaluation of the cultures methane production could not be done.

7.4.1 Discussion of the Results for Experiment 4

The phenol control diverged from the control on day 14. The 40% sample A culture was also slightly higher than the control by this day (14) but it did not diverge significantly until between days 39 and 44.

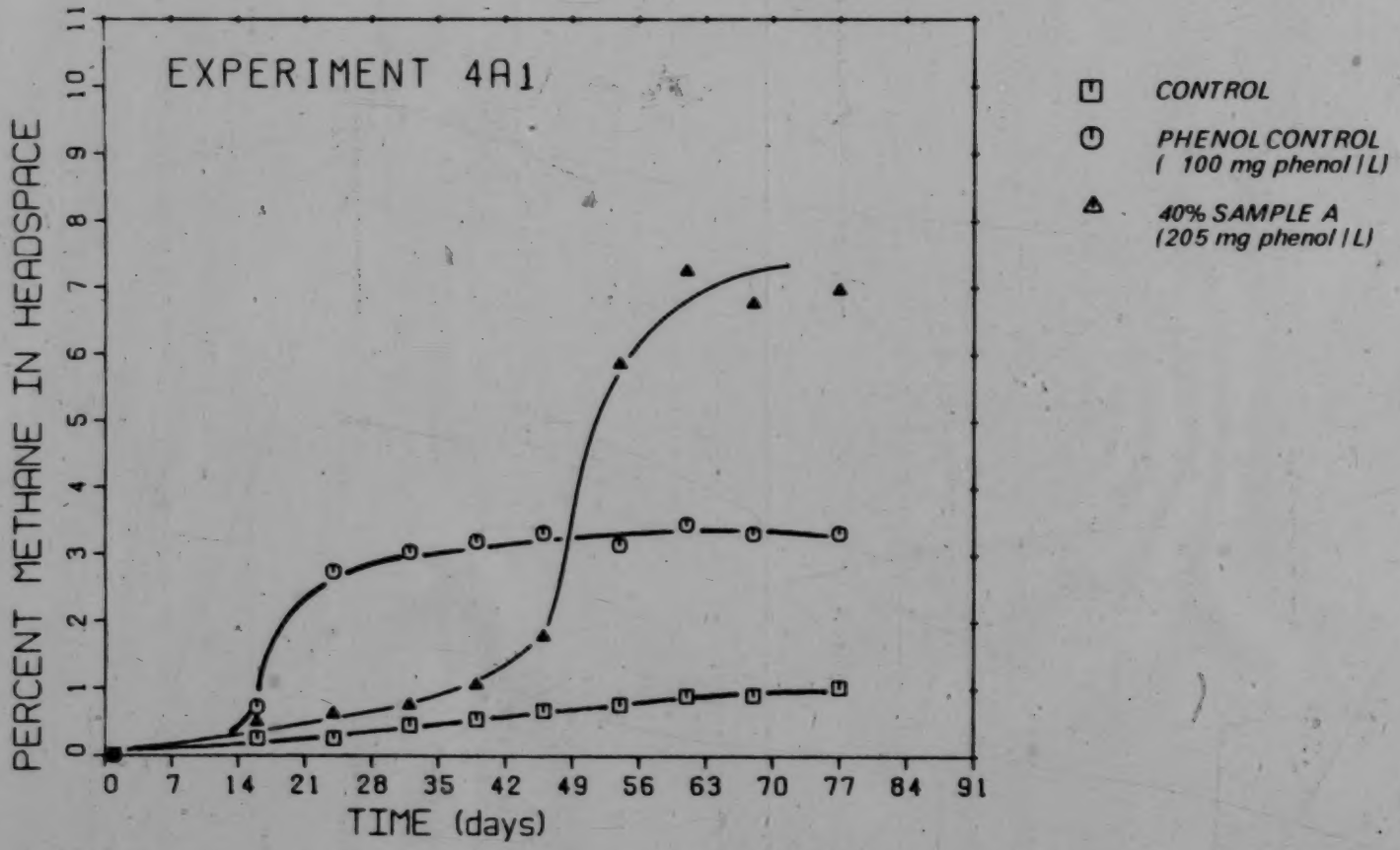
EXPERIMENT 4A1
PERCENT METHANE IN HEADSPACE
0 1 2 3 4 5 6 7 8 9 10 11
0 7 14 21 28 35 42 49 56 63 70 77 84 91
TIME (days)
CONTROL
PHENOL CONTROL
(100 mg phenol / L)
40% SAMPLE A
(205 mg phenol / L)

Figure 15. Adaptive response experiment for previously exhausted N_2 stripped 40% sample A cultures with fresh sample (N_2 stripped and boiled) introduced (draw and feed, single bottle tests).

7.5 Experiment 5

This experiment involved samples A and D. The object was to determine whether sample organic components were inhibitory as well as the inorganic components of samples A and D (as was shown to be the case for sample A in Experiment 2A2). The data for Experiments 5A1 and 5D1 are presented in Figures 16 and 17, and Tables 23, and 24 respectively. Additionally, the effect of boiling sample D as a pretreatment alternative was investigated (Experiment 5D2, and presented in Figure 17 and Table 23).

The same procedure for the organics extraction was followed as in Experiment 2A2 except the organics were back-extracted from the ether phase into an aqueous phase (distilled water). This was achieved by mixing three successive aliquots of distilled water (pH adjusted to 10.0 with 0.1 N NaOH) with the ether solution (240 mL) which presumably contained most of the organics. The left over ether, the inorganic solution and the aqueous solution containing the organics were separately placed on a rotary evaporator.

The sample D boiling pretreatment was carried out according to the previously outlined procedure.

Ammonia was reduced to 400 mg NH_3-N/L from 4200 mg/L (Table 4) for sample D after boiling pretreatment. Sulphide concentrations were less than 50 mg S^{2-}/L for both samples.

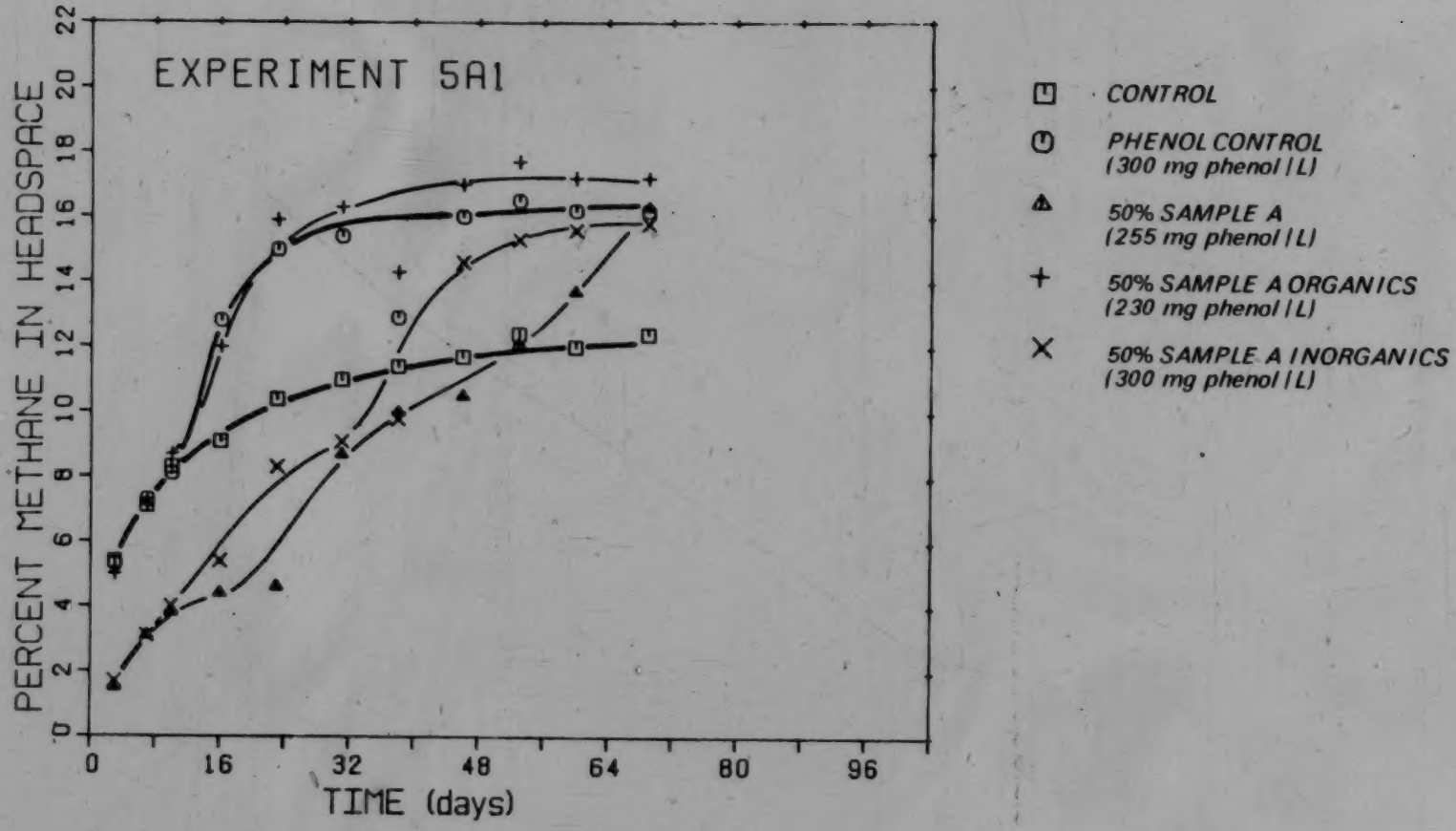

Figure 16. Culture dilutions (50% sample concentrations) with organic and inorganic sample A components. See Table 23.

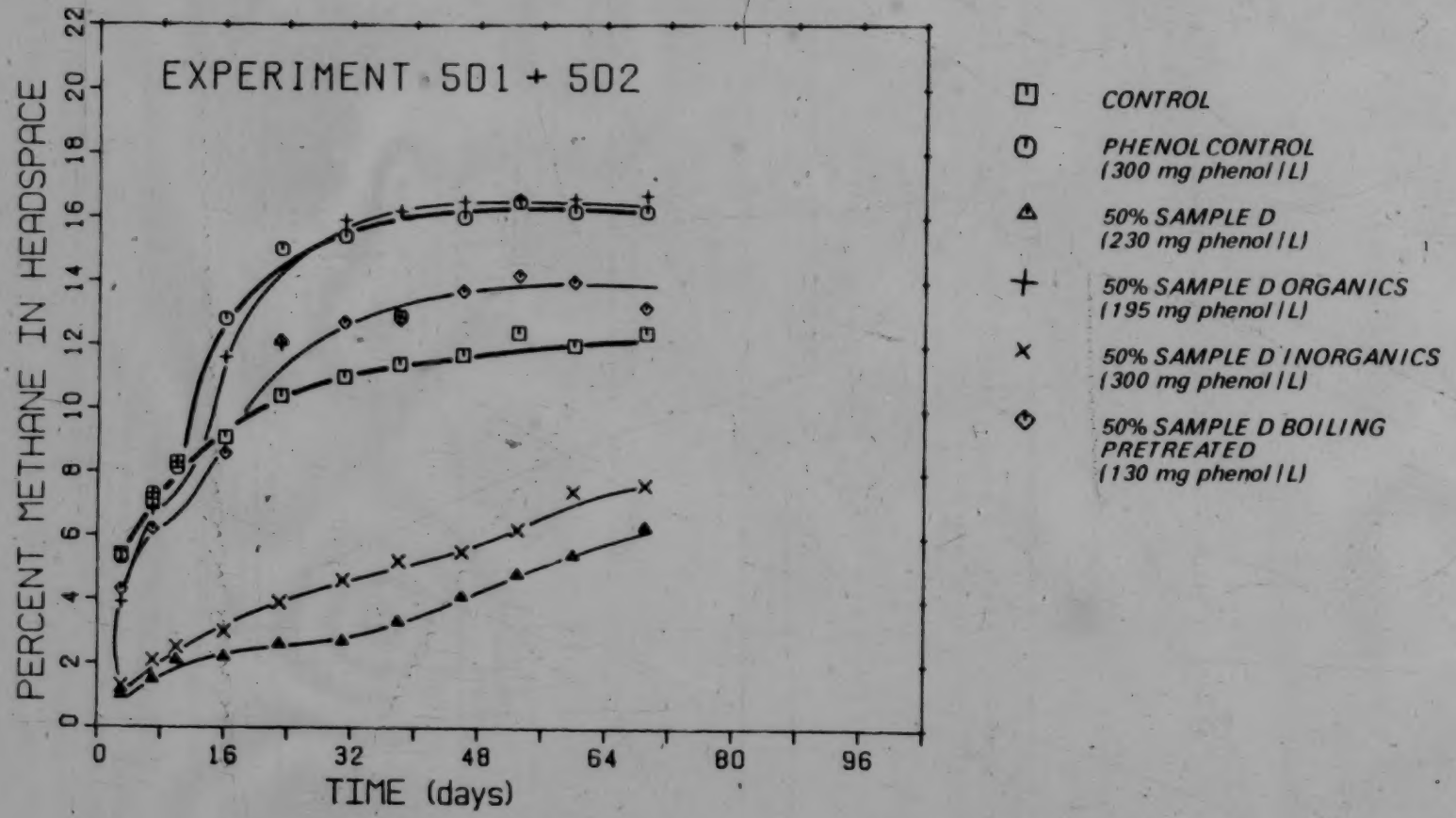

Figure 17. Culture dilutions (50% sample concentrations) for sample D: organic / inorganic solutions and boiling pretreated sample. See Table 24.

7.5.1 Discussion of the Results for Experiment 5

7.5.1.1 Organic/Inorganic Component Extraction Cultures

Both samples A and D showed the same basic trend (Figure 16 and 17); the cultures with the organic components only were not inhibited at all. The curves for these cultures followed the phenol control closely (both had 300 mg phenol/L). On the other hand the inorganic component cultures responded roughly in the same manner as the unaltered 50% sample cultures.

7.5.1.2 Cultures with Boiled Sample D

Boiling sample D as a pretreatment step had beneficial results in reducing inhibiting agents; although the 50% sample D curve (boiling pretreated) never statistically exceeded the control, the mean response did. No inhibition beyond the first sampling day was observed.

7.5.2 Experiment 6

This last experiment involved all the samples. The objective was to further assess the effect of boiling pretreatment. Phenol concentrations for the samples before and after boiling pretreatment are presented in Table 6.

Sample culture dilutions of 30% and 50% were prepared for each sample (Figures 18, 19, 20, and 21, and Tables 25, 26, 27, and 28).

7.5.3 Discussion of the Results for Experiment 6

The results are presented in Figures 18 to 21. Figure 18 indicates that the sample A cultures responded favorably to boiling pretreatment. The 50% culture took slightly longer than the 30% culture to acclimate but the curve eventually exceeded the control, as did the 30% culture.

The sample B cultures (Figure 19) were not inhibited at any time. The difference between the 30% and 50% curves and the control curve were not as pronounced as was the case for the sample A cultures, but this is because of the reduced phenol levels (30 and 60 mg phenol/L for sample B cultures).

No improvement was found for the sample C cultures (Figure 20) after this pretreatment (compared to Figure 3).

The sample D cultures (Figure 21) showed a higher methane response compared to the Experiment 3 (Figure 14) curves (N_2 stripped only), but in this experiment (6D1), the 50% culture was more inhibited than the equivalent one in Experiment 5D2 (Figure 17).

Table 6. Phenol concentrations for samples before and after boiling pretreatment (Experiment 6).

B.P. = before pretreatment
A.P. = after pretreatment

SAMPLE		PHENOL mg/L	% PHENOL REDUCTION
A	B.P.	540	
	A.P.	310	43
B	B.P.	170	
	A.P.	110	35
C	B.P.	140	
	A.P.	90	36
D	B.P.	490	
	A.P.	260	47

Note: The wastewater volume was restored to its original amount after boiling.

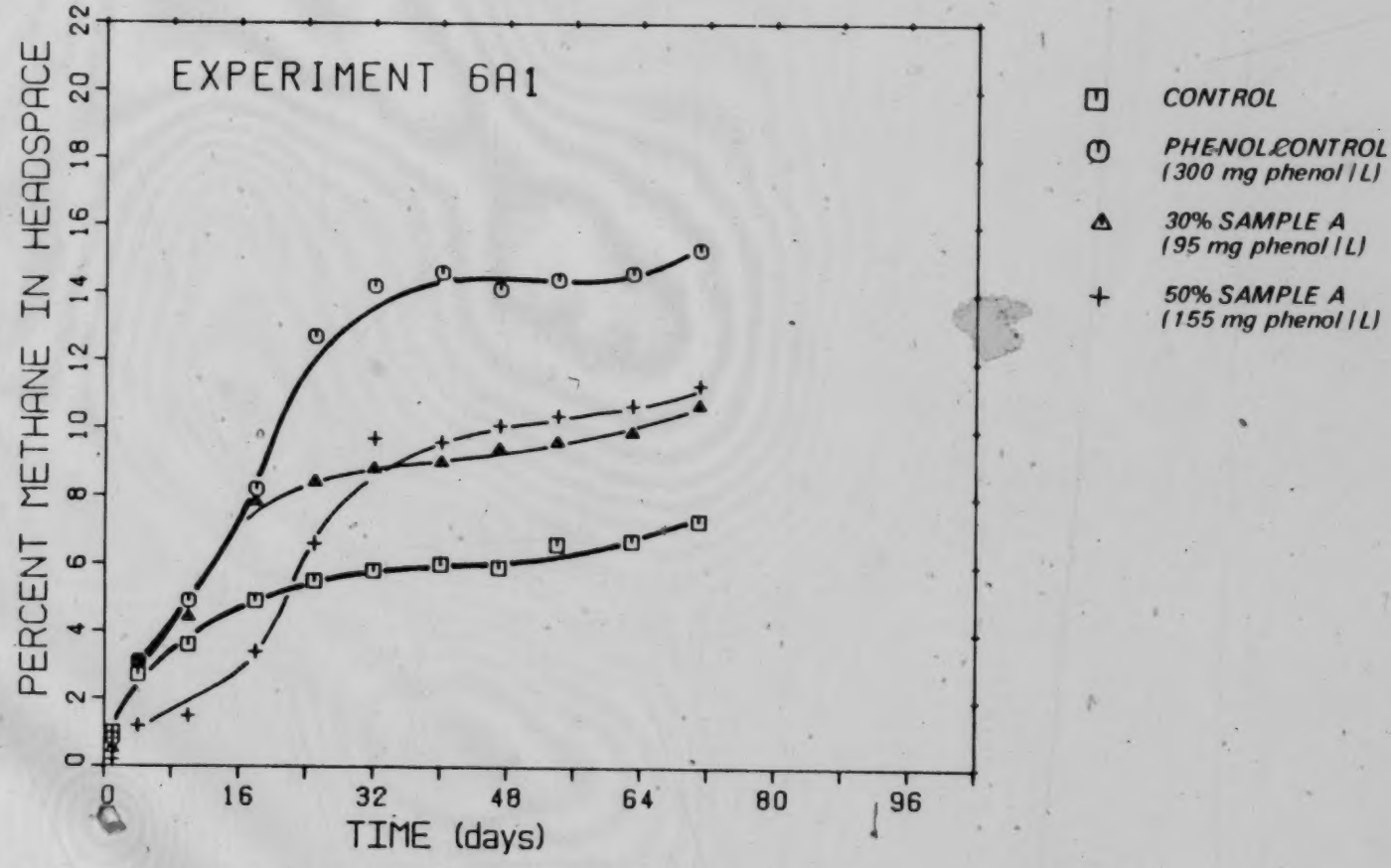

Figure 18. Boiling pretreated sample A culture dilutions. See Table 25.

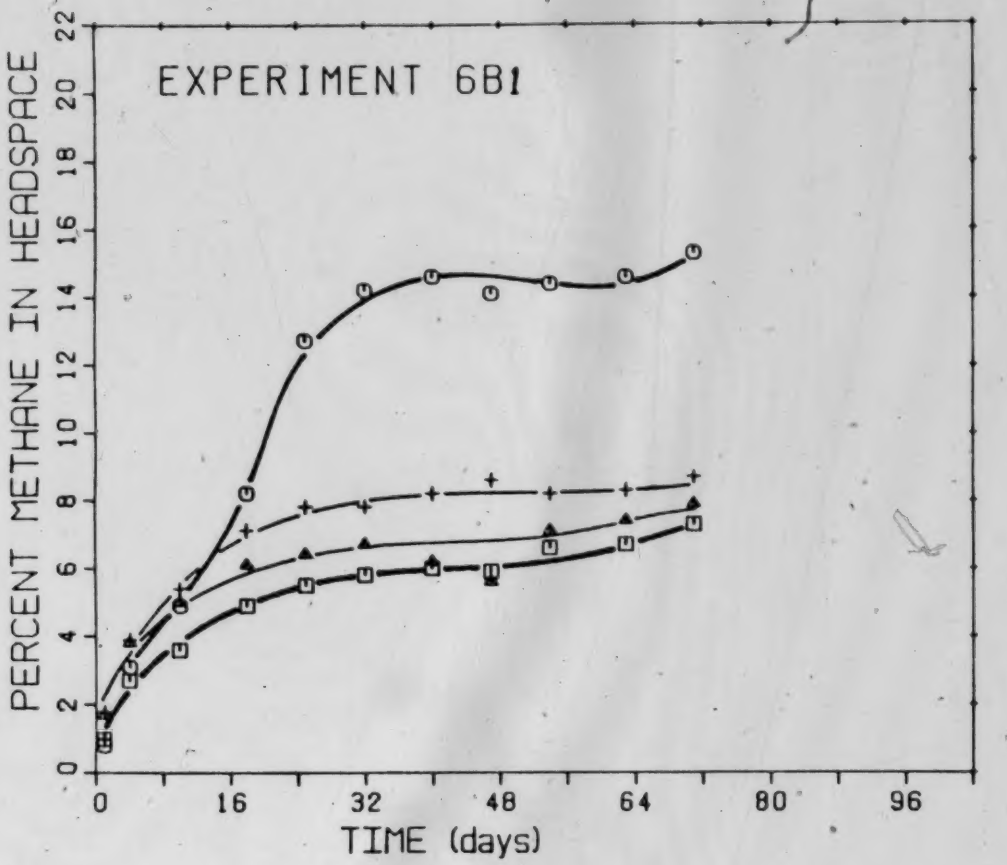

Figure 19. Boiling pretreated sample B culture dilutions. See Table 26.

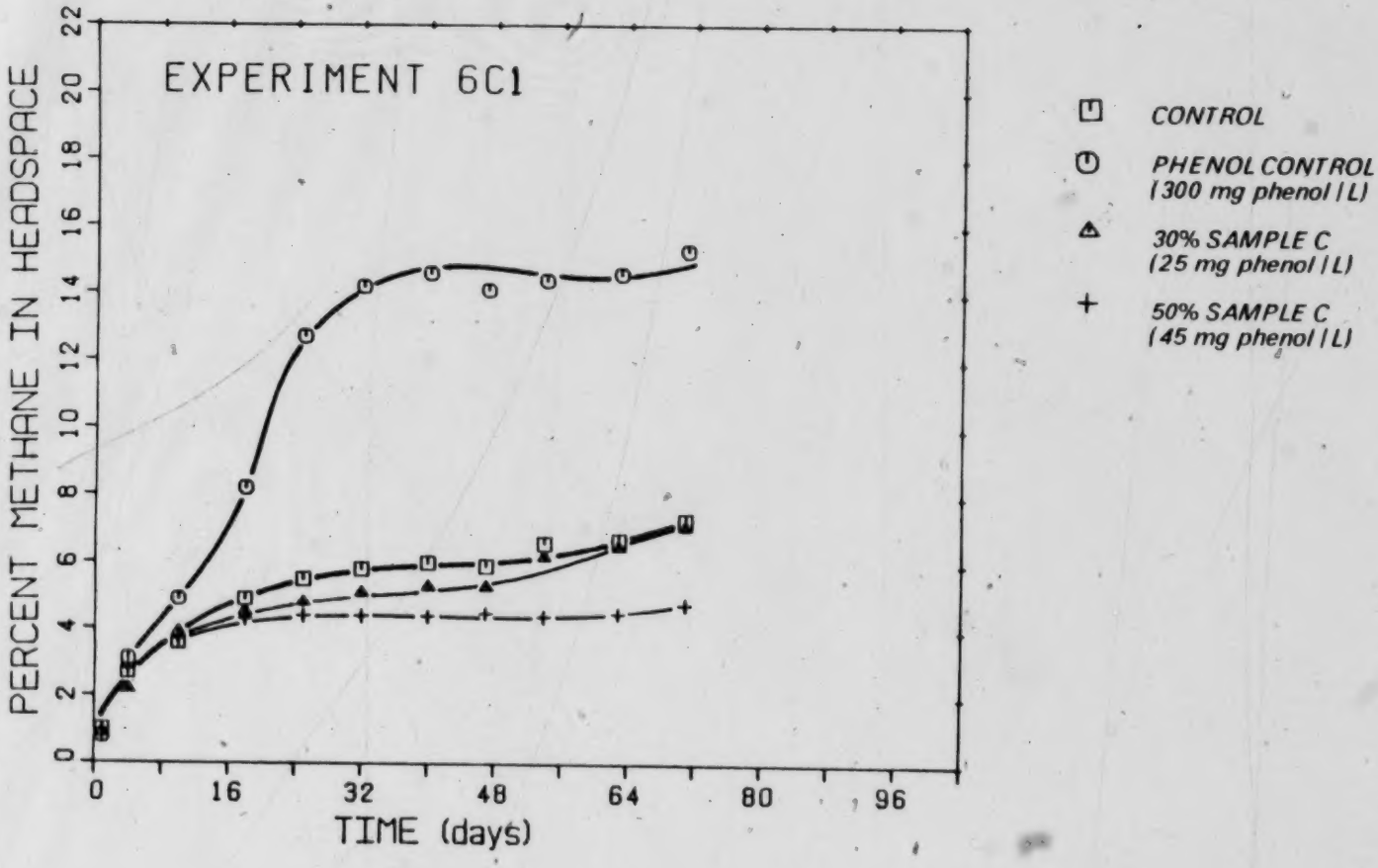

Figure 20. Boiling pretreated sample C culture dilutions. See Table 27.

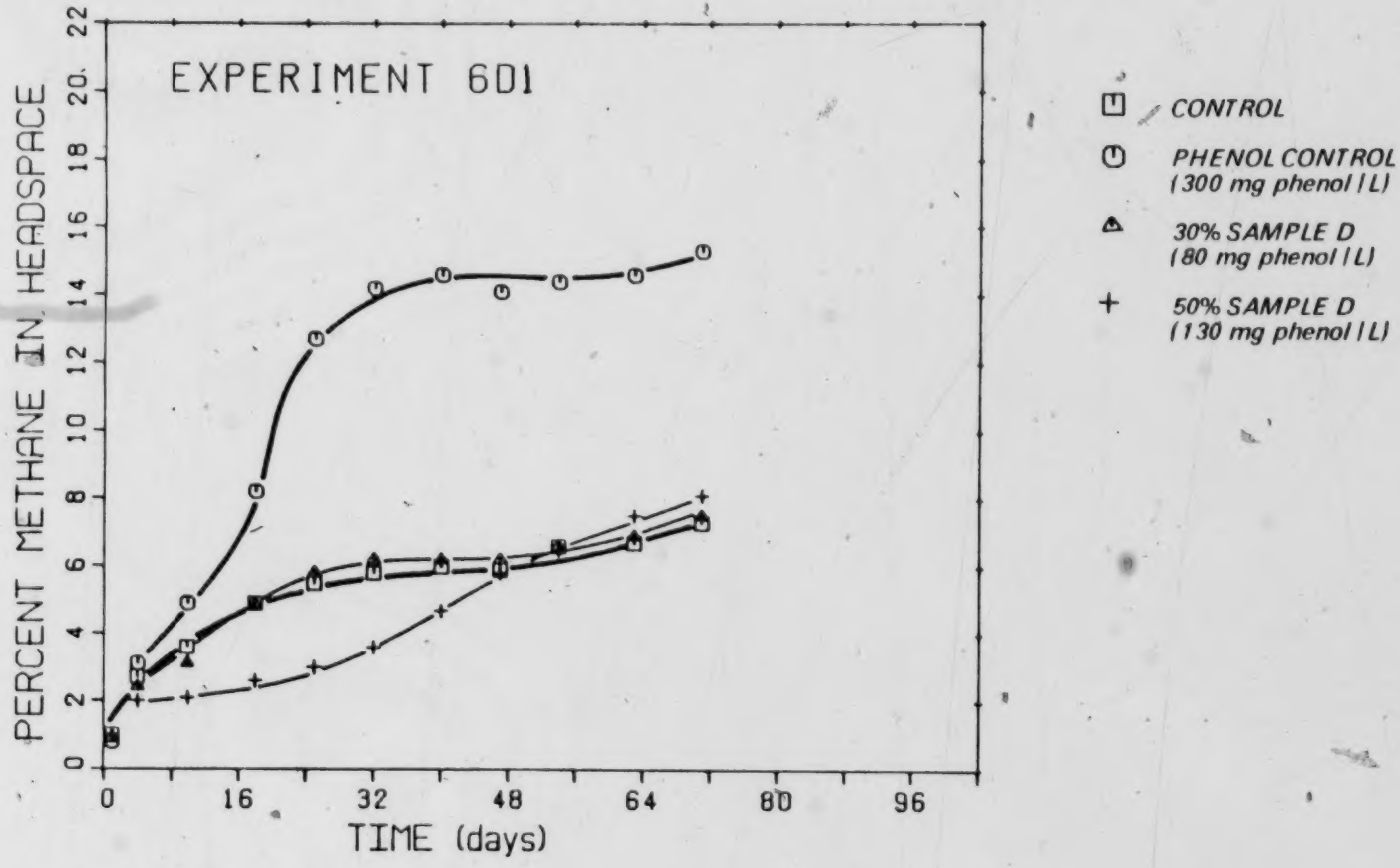

Figure 21. Boiling pretreated sample D culture dilutions. See Table 28.

8. DISCUSSION

Discussion of the results is developed and categorized by addressing the earlier stated research objectives. These objectives are re-summarized for continuity.

- Assess the three pretreatment options - N_2 stripping, batch adsorption with activated carbon, and sample boiling.
- Determine which sample components (organic or inorganic) would exert toxic effects on the cultures.
- Determine if the methanogenic bacterial component was more sensitive to the toxic constituents in the various samples.
- Determine if the bacteria retain the ability to assimilate phenol after the active degradation phase.

The first objective, which was concerned with determining the most effective pretreatment option; specifically, N_2 gas stripping, high temperature pretreatment, and activated carbon pretreatment; shall be separately discussed under the general heading Pretreatment Assessment.

Results pertaining to the last three objectives have been discussed under the heading Inhibition Experiments. The rationale for this categorization is based on the recognition that the various experiments all contributed

information concerning the nature and form of inhibition.

However, before proceeding with the above items, a discussion regarding some variations in the results is warranted.

The methane response curves for some experiments were often higher or lower than those in other experiments (for identical samples and dilutions). This can be explained by the different inoculum sludge solids concentrations for different experiments. Sludge inoculum was usually drawn from the same anaerobic digester at the same draw-off point. Occasionally the sludge was thicker than other times, and sometimes the sludge was so watery looking that a different digester and/or lower draw-off level was used. No attempt was made to duplicate the solids concentration between experiments because a consistent, absolute methane figure was not sought, rather it was the difference between control and sample methane levels that was of interest. The difference from the controls for similar experiments was fairly consistent by the time the curves had reached their plateau.

Another inconsistency involved replicate cultures not responding in the same manner and at the same times for different experiments. For example, Figure 1 and 4 show that the 40% sample A culture began actively fermenting (the mean methane level was statistically above the control non-significance range) on days 49 and 70 for Experiments 1A1 and 2A1 respectively. This, again, may have been due to

the different inocula used. Different numbers, types, and ages of microorganisms would be associated with different sludge samples. The different characteristics and numbers of microorganisms would have a bearing on the ability and time it takes for the microorganisms to acclimate and hence degrade the organics and produce methane.

Sometimes inhibited sample cultures would contribute to a large standard deviation. Often, usually after a lengthy period, one culture (of three in a group) would start responding while the other two would remain inhibited. These inconsistencies may have been caused by the bacteria in one culture being able to acclimate more quickly than the bacteria in other groups, or it may have caused by different numbers of suitable bacteria being present in one bottle and not the other. It appeared that consistent volumes of inoculum were injected; however, if only small numbers of appropriate bacteria were originally present in the sludge, then the inevitable variation in sampling bacteria from the sludge would lead to large relative differences in performance between serum bottles.

8.1 PRETREATMENT ASSESSMENT

The three pretreatment options are discussed under the indicated headings.

8.1.1 Nitrogen Stripping Pretreatment at Room Temperature

Inhibition was found, at least initially, for a majority of the samples tested after this form of pretreatment. The inhibition was most significant for the high sample concentration cultures (30% to 50%).

8.1.1.1 Sample A

A summary of the days that the sample A culture dilutions exhibited methane levels statistically above and below the standard control non-significance range are presented in Table 7. The Dunnett statistical program used generated a lower and upper percent methane figure ($P<0.05$) for each culture, during each sampling day. If the mean culture value (percent methane) falls within this range then the culture in question is judged non-significantly different from the control. These upper and lower percent methane values can be used to compare the progress of replicate cultures. For example, if a culture starts off inhibited and then begins to respond, it is judged not inhibited when its percent methane value exceeds the lower percent methane value generated for the control. When the percent methane value of the culture exceeds the upper percent methane value for the control, the culture is displaying enhanced methane production relative to the control. Table 7 shows, in this way, the progress of Sample A cultures throughout the various experiments.

Table 7. Sample A cultures; enhancement and inhibition statistical data.

	Experiment #							
	1		2		3		5	
DIL.	-	+	-	+	-	+	-	+
10%	NI	11						
20%	NI	11	NI	42	NI	21		
30%	4	23	11	51	43	74		
40%	35	54	59	70	74	NE		
50%	61	101'	91'	NE	96'	NE	46	69'
IP	101		91		96		69	

"-" The day up to which the culture was inhibited.

"+" The day after which the culture exhibited enhanced methane production

IP: incubation period, days

NI: No inhibition

NE: No enhancement

' Last day of sampling

The 10% and 20% sample A cultures were never inhibited, but the higher concentrations required varying acclimation periods (i.e., the 30%, 40%, and 50% sample dilutions). With the exception of the 40% sample culture in Experiment 3A1, the 30% and 40% sample cultures generally indicated enhanced methane production on average after 49 and 62 days respectively.

The 50% sample cultures were constantly inhibited in about half of the experiments (Table 7).

Evidence for phenol degradation in the sample A cultures was obtained by measuring the final phenol concentration after incubation for 3 cultures: Experiment 1A1 40% culture and Experiment 2A1 30% and 40% cultures. The percent phenol reductions were determined to be 94%, 66% and 74% respectively. The 2A1 cultures were incubated for 91 days as opposed to 101 days for the 1A1 cultures; perhaps accounting for some of the difference in percent removal between experiments.

8.1.1.2 Sample B

No inhibition was found for the 10%, 20%, and 30% sample B culture concentrations (Figures 2 and 5). Enhanced methane production occurred almost immediately in these cultures. The 40% and 50% samples indicated inhibition for the first 23 and 33 days respectively. Enhanced methane production did not occur for these dilutions in Experiment 2 (Figure 5).

8.1.1.3 Sample C

Highly variable results were obtained with this sample. Experiment 2 (Figure 6) exhibited enhanced methane production for the 20%, 30%, and 40% sample cultures on days 42, 51, and 70 respectively, while the Experiment 1 cultures (Figure 3) did not exhibit enhanced methane production relative to the control for any of the dilutions. This sample had the least amount of organics present, and this may partially be the reason for the lack of enhanced methane production.

8.1.1.4 Sample D

In spite of this sample having originated from the same source as sample A, it was found to be much more toxic to the culture microorganisms. All of the cultures (Figures 14, 17, and 21) were inhibited for some period. Enhanced methane production occurred only for the 20% and 30% sample cultures (Figure 14), but only on days 96 and 89 respectively (Table 22).

Summary

The N_2 stripping method of pretreatment was not successful in preventing culture inhibition for the higher concentration sample dilutions (30% to 50%), but did allow methanogenesis for many of the samples at lower sample concentrations.

8.1.2 Batch Adsorption with Activated Carbon Pretreatment

This pretreatment alternative was marginally beneficial for the sample cultures. This finding is consistent with the results of the inorganic/organic component experiments, which showed that it was the inorganic constituents that were causing inhibition. Activated carbon is generally not very effective in adsorbing inorganic constituents.

Experiments 2A3, 2B3, and 2C3 (Figures 7, 8, and 9) involved using several different dosages of activated carbon (only the 10 g A.C./L curve is presented in the figures; Tables 15, 16, and 17 (in Appendix B) have the other concentrations). Only the highest dosage, 10 g/L, had any positive effect on the sample A cultures, but no effect was detected for sample C, even at this dosage. The sample A culture exhibited a marginal positive response (i.e., the inhibition period was reduced from 91 days to 82 days).

Experiment 3A2 (Figure 13) involved pretreating the sample with 10 g/L activated carbon, using a 7 day contact time. This had the effect of reducing the 50% sample culture inhibition time by half and the 40% culture inhibition time from 74 to 7 days (see Table 22 for 40% data).

8.1.2.1 Summary

The batch adsorption with activated carbon pretreatment only permitted a significant reduction in culture inhibition when the sample was pretreated using very long contact times (7 days), and high activated carbon dosages. Otherwise activated carbon was found to

have only marginal benefit in reducing toxicity.

8.1.3 Boiling Pretreatment

As discussed earlier, the boiling pretreatment was probably more severe than that found for refinery stripping columns (Table 6). In practice, maximum phenol reductions are reported to be no greater than 35% (American Petroleum Institute 1969). With a reduced substrate concentration it was difficult to discern positive responses from the sample cultures relative to the control cultures. In spite of this disadvantage it was found that this pretreatment alternative permitted significant shortening of the acclimation periods for most cultures.

The first time this pretreatment was used was for sample D in Experiment 5D2 (Figure 17); it reduced the acclimation time for the 50% sample D culture from 53 to 7 days. In a later experiment, 6D1, no inhibition was detected. Acclimation times for 50% culture dilutions of sample A, B, and C, after this pretreatment, were 18, 0, and 71 days respectively. Sample C was found to be notably resistant to anaerobic treatment, regardless of the pretreatment method employed.

8.1.3.1 Summary

High temperature pretreatment reduced inhibition times for most of the samples. However, the pretreatment operation was severe, removing a major portion of phenol. Further research should determine the degree of

pretreatment necessary to eliminate toxicity with a minimum reduction in organics.

8.2 CULTURE INHIBITION EXPERIMENTS

As the results for the various pretreatment alternatives indicated, inhibition occurred for all of the samples, at many of the dilutions, even after boiling pretreatment.

The other aspect of this research was to try to determine the toxicants responsible for inhibition, and the effect they have on the specific bacterial components.

8.2.1 Organic/Inorganic Component Separation Experiments

These experiments were designed to provide information regarding the specific constituents, organic or inorganic, that would cause culture inhibition.

The organic components were separated using diethlyether as an extractive solvent. The resulting inorganic solution was spiked with phenol to provide a culture substrate.

All of the inorganic component cultures were about as inhibitory as the unaltered 50% sample cultures (Figures 7, 8, 9, 16, and 17). None of the organic component cultures (Figures 16 and 17) were found to be inhibitory at any time and they usually responded with enhanced methane production at about the same time as the phenol control, indicating unhindered microorganism adaptation to phenol.

Although the results must be carefully interpreted because of the inadequacies of the extraction procedures, they appear to have indicated that an inorganic constituent or constituents were responsible for inhibition in all of the cultures. The batch adsorption with activated carbon experiments support this interpretation. There is some question as to which inorganic constituent could be responsible for this inhibition. Ammonia nitrogen must be ruled out as a toxicant since the maximum tolerable concentrations have been found (Parkin and Miller 1982, see section 3.6) to be much higher than the sample ammonia nitrogen concentrations (Table 4).

It is also doubtful that ions, such as sodium or chloride, would have been present in high enough concentrations to cause inhibition. However, it is possible that cyanide or heavy metals in the samples may have inhibited the cultures. The heavy metals may have precipitated during boiling pretreatment (a heavy precipitate was reported in Section 6.2.3), and the cyanide may have escaped the solution in the gaseous phase after the boiling pretreatment.

8.2.1.1 Summary

The results, supported by the results of the batch adsorption with activated carbon pretreatment experiments, indicate that inorganic constituents are responsible for inhibition in the cultures.

8.2.2 Acetate Enrichment Experiments

The object of these experiments (Experiments 2A4, 2B4, and 2C4) was to determine whether one, or both of the main bacterial groups (acid formers and methanogens) were inhibited. Acetate is a methanogen specific substrate.

Although none of the acetate spiked 50% cultures (samples A, B, and C, in Figures 10, 11, and 12) responded immediately to the added acetate, they all did so within 20 days (relative to the unaltered 50% cultures), indicating that the methanogens were less inhibited than the acid formers.

The acetate spiked (750 mg acetate/L) cultures did not indicate that phenol was being degraded since the acetate spiked culture did not exceed the acetate control; at least for the duration of the experiment.

8.2.2.1 Summary

The methanogens were shown to be less inhibited than the acid forming bacterial component.

8.2.3 Microorganism Adaptive Response

This experiment (Experiment 4, Figure 15) was designed to provide information on the ability of the microorganisms to remain adapted to phenol (pure and sample phenol) after the active phenol degradation phase had passed (when the curve had reached a plateau).

8.2.3.1 Summary

The experiment clearly indicated that if the bacteria are not in an active phase of degradation, they must re-acclimate to the synthetic phenol and sample organics.

9. CONCLUSION

The conclusions are summarized as follows:

- Boiling pretreatment was the most effective pretreatment method for reducing culture inhibition.
- Inorganic constituent(s) appear to cause inhibition. This conclusion is supported by the results of the batch adsorption with activated carbon pretreatment experiments.
- The methanogens were found to be less sensitive to the sample toxicants than the acid forming bacteria.
- The culture microorganisms did not remain adapted for phenol assimilation after initial acclimation.

In general, considerable variation in methane responses for the different sour water samples was observed. This variation was to be expected considering each sour water sample was associated with different contributing refinery processes and feedstocks. Unexpected, however, was the variation found for the same samples in different experiments. This may have been partially attributable to the different inocula used for the experiments.

10. RECOMMENDATIONS

This research can only be considered as a preliminary assessment of the anaerobic biological treatability of refinery sour water. Further research should identify the specific toxic constituent(s) in the various sour water samples. Also, it is important to more precisely define the boiling pretreatment times necessary to render the samples non-toxic. Finally, semi-continuous feed experiments should be conducted to determine the detention times necessary to reduce the sample organics to acceptable levels, and to assess the possibility of toxicants building up in the cultures.

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APPENDIX A

Table 8, Phenol determinations[1]

CONDITION	SAMPLE PHENOL (mg/L) A	B	C	D
Raw	540	170	140	490
N_2 Stripped	510	160	140	460
Organic Solution[2]	450	na	na	390
Inorganic Solution[2]	0	0	0	10
Boiled	310	110	90	260
A.C.				
10 g/L	10	0	1	na
4 g/L	130	10	10	na
1 g/L	380	100	70	na
Terminal Culture Conc.[3]				
30%(Expt.2)	52	nd	nd	nd
40%(Expt.1)	13	nd	nd	nd
40%(Expt.2)	54	nd	nd	nd

na = not applicable

nd = not determined

[1] as described in Section 5.2.2

[2] volume of solution equivalent to original sample volume

[3] final phenol concentration in cultures (culture solutions filtered through a Whatman #1 filter paper. The Experiment 1 incubation time was 101 days and Experiment 2 incubation time was 91 days.

APPENDIX B

This appendix contains tables only. These tables contain the statistical information supporting the data comprising the figures in the text. As well, they contain data not included in the figures.

Table 9. Nitrogen stripped sample A culture dilutions (Experiment 1A1).

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
	PERCENT METHANE IN HEADSPACE							
1	1.8	1.9	1.9	1.0	0.6	0.6	0.5	1.1-2.5
std. dev.	*0.2*	*0.4*	*0.5*	*0.3*	*0.2*	*0.1*	*0.2*	
2	2.3	2.6	3.0	2.6	1.0	0.8	0.6	1.3-3.3
std. dev.	*0.2*	*0.3*	*0.5*	*0.8*	*0.5*	*0.2*	*0.2*	
3	2.9	3.1	2.6	4.0	1.7	1.1	0.7	1.8-4.0
std. dev.	*0.2*	*0.2*	*2.4*	*0.2*	*0.7*	*0.3*	*0.2*	
4	3.2	3.4	4.0	4.3	2.2	1.4	0.8	2.3-4.0
std. dev.	*0.2*	*0.3*	*0.3*	*0.2*	*0.7*	*0.3*	*0.3*	
5	3.5	3.7	4.3	4.6	2.7	1.8	0.9	2.6-4.4
std. dev.	*0.2*	*0.2*	*0.3*	*0.2*	*0.7*	*0.3*	*0.1*	
6	3.8	4.1	4.6	4.9	3.3	2.0	1.1	2.7-4.9
std. dev.	*0.2*	*0.2*	*0.3*	*0.2*	*0.9*	*0.2*	*0.5*	
7	4.2	4.4	4.9	5.0	4.0	2.0	1.3	2.9-5.4
std. dev.	*0.2*	*0.2*	*0.3*	*0.1*	*1.2*	*0.2*	*0.6*	
9	4.5	4.8	5.5	5.5	4.8	2.2	1.6	3.5-5.5
std. dev.	*0.2*	*0.2*	*0.4*	*0.2*	*0.7*	*0.2*	*0.6*	
11	4.9	5.4	6.5	6.2	5.5	2.3	1.8	3.8-6.0
std. dev.	*0.2*	*0.2*	*0.7*	*0.3*	*0.6*	*0.2*	*0.6*	
13	5.5	6.5	7.5	7.6	6.0	2.5	1.9	4.4-6.6
std. dev.	*0.2*	*0.3*	*0.5*	*0.5*	*0.7*	*0.2*	*0.5*	
15	5.6	7.4	7.8	9.1	6.3	2.4	1.8	4.2-7.0
std. dev.	*0.1*	*0.2*	*0.5*	*0.8*	*1.2*	*0.3*		
17	6.0	9.1	8.2	10.1	7.0	2.6	1.9	4.4-7.6
std. dev.	*0.1*	*0.2*	*0.6*	*0.2*	*1.4*	*0.3*	*0.5*	

Table 9 continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
20	6.3	12.8	8.5	10.8	8.7	2.8	2.0	3.8-8.7
std. dev.	*0.1*	*0.9*	*0.6*	*0.2*	*2.3*	*0.4*	*0.5*	
23	6.5	16.1	8.6	11.0	10.7	3.1	2.1	4.2-8.8
std. dev.	*0.1*	*1.6*	*0.6*	*0.2*	*2.0*	*0.4*	*0.5*	
26	7.1	17.1	9.1	11.3	12.1	3.8	2.5	5.8-8.3
std. dev.	*0.1*	*0.5*	*0.6*	*0.2*	*0.8*	*0.5*	*0.4*	
30	7.3	17.9	9.5	11.2	12.8	4.1	2.4	5.7-8.9
std. dev.	*0.2*	*0.3*	*0.7*	*0.7*	*1.0*	*0.7*	*0.5*	
35	7.5	18.5	9.6	11.7	13.1	5.4	2.5	6.0-9.1
std. dev.	*0.1*	*0.3*	*0.6*	*0.4*	*0.9*	*1.1*	*0.5*	
39	7.4		9.1	11.6	12.5	5.7	2.7	5.7-9.1
std. dev.	*0.1*		*0.5*	*0.2*	*0.9*	*1.2*	*0.6*	
44	7.9		10.0	12.2	13.4	8.5	3.4	5.6-10.2
std. dev.	*0.2*		*0.6*	*0.2*	*0.8*	*1.9*	*0.5*	
49	7.9		10.0	12.1	13.3	11.2	4.6	4.7-11.2
std. dev.	*0.3*		*0.7*	*0.1*	*0.8*	*3.5*	*0.4*	
54	7.9		10.4	12.4	13.6	13.8	5.4	5.3-10.5
std. dev.	*0.3*		*0.8*	*0.3*	*0.9*	*2.3*	*0.3*	
61	8.4		11.1	13.2	14.4	16.8	6.4	6.8-10.0
std. dev.	*0.4*		*0.7*	*0.1*	*1.1*	*0.4*	*0.8*	
70	8.5		11.2	13.3	14.8	17.6	7.3	6.7-10.3
std. dev.	*0.5*		*0.7*	*0.3*	*1.3*	*0.1*	*0.9*	
78	8.8	21.6	11.7	14.9	15.7	19.3	9.0	6.4-11.3
std. dev.	*0.6*	*1.4*	*0.8*	*0.6*	*1.3*	*0.1*	*1.4*	

Table 9 continued

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
			PERCENT METHANE IN HEADSPACE					
89	9.2		11.9	14.0	14.7	18.0	13.2	5.0-13.5
std. dev.	*0.5*		*1.1*	*0.3*	*0.7*	*0.1*	*4.0*	
101	9.0	20.8	11.9	14.1	15.5	19.1	18.0	6.2-11.9
std. dev.	*0.7*	*1.4*	*1.0*	*0.4*	*1.2*	*0.1*	*2.1*	

Table 10. Nitrogen stripped sample B culture dilutions (Experiment 181).

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
	PERCENT METHANE IN HEADSPACE							
1	1.8	1.9	3.3	3.6	1.7	1.3	1.0	0.9-2.7
std. dev.	*0.2*	*0.4*	*0.5*	*0.5*	*0.5*	*0.2*	*0.1*	
2	2.3	2.6	3.8	4.3	2.2	1.6	1.2	1.5-3.1
std. dev.	*0.2*	*0.3*	*0.4*	*0.1*	*0.6*	*0.2*	*0.2*	
3	2.9	3.1	4.3	4.8	2.6	1.9	1.5	2.3-3.6
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.4*	*0.2*	*0.2*	
4	3.2	3.4	4.6	5.0	2.9	1.9	1.6	2.6-3.8
std. dev.	*0.2*	*0.3*	*0.4*	*0.1*	*0.3*	*0.2*	*0.2*	
5	3.5	3.7	4.8	5.2	3.3	1.9	1.6	3.0-4.0
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.1*	*0.2*	*0.2*	
6	3.8	4.1	5.1	5.4	4.1	2.0	1.6	3.3-4.4
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.2*	*0.2*	*0.2*	
7	4.2	4.4	5.4	5.6	5.0	2.0	1.6	3.5-4.8
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.4*	*0.2*	*0.2*	
9	4.5	4.8	5.8	6.2	5.7	2.1	1.6	4.1-5.0
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.2*	*0.2*	*0.2*	
11	4.9	5.4	6.3	6.9	6.1	2.3	1.7	4.4-5.5
std. dev.	*0.2*	*0.2*	*0.4*	*0.1*	*0.2*	*0.2*	*0.2*	
13	5.5	6.5	6.7	7.4	6.5	2.5	1.8	4.9-6.1
std. dev.	*0.2*	*0.3*	*0.5*	*0.1*	*0.2*	*0.1*	*0.2*	
15	5.6	7.4	6.7	7.5	6.9	2.6	1.6	5.0-6.3
std. dev.	*0.1*	*0.2*	*0.5*	*0.1*	*0.3*	*0.1*	*0.2*	
17	6.0	9.1	7.0	7.8	7.8	3.2	1.7	5.4-6.6
std. dev.	*0.1*	*0.2*	*0.5*	*0.1*	*0.3*	*0.1*	*0.2*	

Table 10. continued

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
			PERCENT METHANE IN HEADSPACE					
20	6.3	12.8	7.4	8.2	8.6	4.3	1.8	5.6-6.9
std. dev.	*0.1*	*0.9*	*0.5*	*0.1*	*0.2*	*0.2*	*0.2*	
23	6.5	16.1	7.5	8.3	8.7	5.8	2.1	5.8-7.2
std. dev.	*0.1*	*1.6*	*0.5*	*0.1*	*0.2*	*0.4*	*0.3*	
26	7.1	17.1	8.0	8.7	8.8	6.6	2.8	5.6-8.5
std. dev.	*0.1*	*0.5*	*0.4*	*0.2*	*0.2*	*1.3*	*0.4*	
30	7.3	17.9	8.3	9.1	9.1	7.7	3.4	6.5-8.2
std. dev.	*0.2*	*0.3*	*0.5*	*0.1*	*0.1*	*0.2*	*0.6*	
35	7.5	18.5	8.0	9.4	7.3	7.5	5.5	3.3-11.7
std. dev.	*0.1*	*0.3*	*0.9*	*0.1*	*3.6*	*0.9*	*1.7*	
39	7.4		8.3	9.0	9.4	7.7	6.9	6.5-8.3
std. dev.	*0.1*		*0.4*	*0.2*	*0.3*	*1.4*	*0.6*	
44	7.9		8.9	9.6	10.0	9.0	8.0	7.3-8.62
std. dev.	*0.2*		*0.4*	*0.1*	*0.4*	*0.1*	*0.3*	
49	7.9		9.1	9.8	10.1	10.5	8.6	7.2-8.62
std. dev.	*0.3*		*0.4*	*0.1*	*0.3*	*0.1*	*0.4*	
54	7.9		9.1	9.9	10.3	11.2	10.3	6.8-9.05
std. dev.	*0.3*		*0.5*	*0.2*	*0.4*	*0.2*	*0.9*	
61	8.4		9.6	10.5	10.9	11.8	11.9	7.4-9.40
std. dev.	*0.4*		*0.6*	*0.2*	*0.4*	*0.1*	*0.5*	
70	8.5		9.8	10.9	11.2	11.9	12.8	7.6-9.53
std. dev.	*0.5*		*0.6*	*0.3*	*0.3*	*0.2*	*0.3*	
78	8.8		10.1	11.5	11.7	12.4	13.6	7.5-10.1
std. dev.	*0.6*		*0.8*	*0.3*	*0.5*	*0.1*	*0.7*	

Table 10. continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
89	9.2		10.7	11.9	12.1	12.9	13.4	7.9-10.6
std. dev.	*0.5*		*0.2*	*0.2*	*0.5*	*0.1*	*0.7*	
101	9.0		10.1	11.8	12.2	12.8	13.7	7.5-10.5
std. dev.	*0.7*		*0.9*	*0.4*	*0.6*	*0.3*	*0.6*	

Table 11. Nitrogen stripped sample C culture dilutions (Experiment 1C1).

	PERCENT METHANE IN HEADSPACE							
DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.8	1.9	2.4	1.8	2.3	0.7	0.5	0.8-2.9
std. dev.	*0.2*	*0.4*	*0.5*	*0.8*	*0.3*	*0.2*	*0.2*	
2	2.3	2.6	3.9	2.9	3.4	1.2	0.7	1.2-3.4
std. dev.	*0.2*	*0.3*	*0.5*	*0.9*	*0.2*	*0.4*	*0.2*	
3	2.9	3.1	3.7	3.7	4.3	1.9	1.1	1.9-4.0
std. dev.	*0.2*	*0.2*	*0.4*	*0.6*	*0.1*	*0.7*	*0.3*	
4	3.2	3.4	4.0	4.0	4.5	2.1	1.2	2.0-4.3
std. dev.	*0.2*	*0.3*	*0.4*	*0.6*	*0.1*	*0.8*	*0.3*	
5	3.5	3.7	4.3	4.2	4.7	2.5	1.3	3.0-4.0
std. dev.	*0.3*	*0.2*	*0.3*	*0.6*	*0.1*	*1.0*	*0.3*	
6	3.8	4.1	4.6	4.5	4.9	2.9	1.4	2.4-5.2
std. dev.	*0.2*	*0.2*	*0.3*	*0.6*	*0.1*	*1.2*	*0.4*	
7	4.2	4.4	4.8	4.6	5.0	3.2	1.4	2.6-5.7
std. dev.	*0.2*	*0.2*	*0.3*	*0.6*	*0.2*	*1.3*	*0.4*	
9	4.5	4.8	5.2	4.9	5.3	3.7	1.6	2.8-6.3
std. dev.	*0.2*	*0.2*	*0.3*	*0.6*	*0.1*	*1.5*	*0.5*	
11	4.9	5.4	5.7	5.2	5.5	4.1	1.7	3.1-6.8
std. dev.	*0.2*	*0.2*	*0.4*	*0.6*	*0.2*	*1.6*	*0.5*	
13	5.5	6.5	6.2	5.5	5.8	4.4	1.9	3.6-7.4
std. dev.	*0.2*	*0.3*	*0.4*	*0.6*	*0.2*	*1.7*	*0.6*	
15	5.6	7.4	6.4	5.6	5.9	4.4	1.8	3.7-7.6
std. dev.	*0.1*	*0.2*	*0.4*	*0.6*	*0.2*	*1.7*	*0.7*	
17	6.0	9.1	6.7	5.9	6.2	4.6	1.9	4.0-8.0
std. dev.	*0.1*	*0.2*	*0.4*	*0.7*	*0.2*	*1.7*	*0.7*	

Table 11. continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
20	6.3	12.8	7.0	6.4	6.4	4.8	2.0	4.3-8.3
std. dev.	*0.1*	*0.9*	*0.4*	*0.6*	*0.2*	*1.7*	*0.8*	
23	6.5	16.1	7.1	6.5	6.5	4.9	2.2	4.5-8.5
std. dev.	*0.1*	*1.6*	*0.4*	*0.7*	*0.2*	*1.7*	*0.8*	
26	7.1	17.1	7.6	7.0	7.0	5.4	2.6	5.0-9.1
std. dev.	*0.1*	*0.5*	*0.3*	*0.7*	*0.2*	*1.7*	*0.9*	
30	7.3	17.9	7.9	6.9	6.8	5.5	2.5	5.2-9.4
std. dev.	*0.2*	*0.3*	*0.4*	*0.5*	*0.1*	*1.7*	*1.0*	
35	7.5	18.5	8.1	6.7	5.7	3.9	2.0	5.0-10.0
std. dev.	*0.1*	*0.3*	*0.3*	*0.4*	*1.5*	*1.8*	*0.7*	
39	7.4		7.9	7.1	7.0	3.9	2.5	4.6-10.2
std. dev.	*0.1*		*0.4*	*0.8*	*0.3*	*2.5*	*1.0*	
44	7.9		8.3	7.8	7.5	5.8	2.7	5.6-10.2
std. dev.	*0.2*		*0.4*	*1.0*	*0.6*	*1.5*	*1.1*	
49	7.9		8.5	8.1	7.8	6.1	1.2	5.6-10.2
std. dev.	*0.3*		*0.4*	*1.0*	*0.9*	*1.4*	*1.2*	
54	7.9		8.6	8.2	7.9	6.2	3.3	5.4-10.5
std. dev.	*0.3*		*0.3*	*1.0*	*1.2*	*1.4*	*1.4*	
61	8.4		8.9	8.7	8.3	6.5	3.9	5.4-11.4
std. dev.	*0.4*		*0.5*	*0.8*	*1.4*	*1.4*	*2.0*	
70	8.5		9.3	9.2	8.7	7.1	5.0	5.8-11.2
std. dev.	*0.5*		*0.3*	*0.7*	*1.1*	*1.3*	*2.0*	
78	8.8		9.7	9.6	9.3	7.9	6.6	6.9-10.8
std. dev.	*0.6*		*0.4*	*0.6*	*0.8*	*1.2*	*0.8*	

Table 11. continued

DAY	CONTROL	PHENOL CONTROL	PERCENT METHANE IN HEADSPACE 10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
89	9.2		10.1	10.2	10.6	9.1	7.5	7.5-11.0
std. dev.	*0.5*		*0.3*	*0.6*	*0.1*	*1.2*	*0.8*	
101	9.0		9.8	10.0	10.3	9.2	7.7	7.2-10.8
std. dev.	*0.7*		*0.1*	*0.7*	*0.2*	*1.4*	*0.7*	

Table 12. Nitrogen stripped sample A culture dilutions (Experiment 2A1).

DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
			PERCENT METHANE IN HEADSPACE				
1	1.7	1.7	0.5	0.5	0.4	0.4	
std. dev.	*0.4*	*0.4*	*0.1*	*0.1*	*0.1*	*0.2*	
2	2.5	2.6	1.7	1.1	0.6	0.5	1.4-3.6
std. dev.	*0.3*	*0.3*	*0.8*	*0.3*	*0.2*	*0.3*	
3	3.2	3.2	2.4	1.5	0.8	0.6	1.8-4.5
std. dev.	*0.4*	*0.4*	*0.9*	*0.6*	*0.3*	*0.5*	
4	3.7	3.7	3.3	1.9	1.0	0.7	2.3-5.2
std. dev.	*0.4*	*0.3*	*0.9*	*0.7*	*0.4*	*0.5*	
5	4.1	4.0	3.7	2.1	1.3	0.9	2.4-5.8
std. dev.	*0.4*	*0.3*	*0.9*	*0.8*	*0.5*	*0.9*	
7	4.5	4.4	4.1	2.3	1.4	1.1	2.8-6.2
std. dev.	*0.3*	*0.3*	*0.9*	*0.8*	*0.4*	*0.9*	
9	4.7	4.7	4.5	2.4	1.6	1.4	3.1-6.4
std. dev.	*0.4*	*0.3*	*0.8*	*0.9*	*0.4*	*0.8*	
11	4.9	4.7	4.7	2.5	1.6	1.5	3.5-6.3
std. dev.	*0.4*	*0.1*	*0.3*	*1.0*	*0.3*	*0.7*	
15	5.2	5.2	6.0	3.0	1.8	1.7	2.6-7.9
std. dev.	*0.4*	*0.3*	*1.7*	*1.6*	*0.3*	*0.5*	
20	5.5	5.9	6.8	3.4	1.8	2.0	2.6-8.4
std. dev.	*0.3*	*0.4*	*1.8*	*1.9*	*0.3*	*0.3*	
25	5.9	7.1	7.9	3.0	2.0	2.2	3.0-8.9
std. dev.	*0.2*	*0.6*	*1.7*	*2.1*	*0.4*	*0.3*	
30	6.5	9.4	9.4	5.4	2.2	2.4	3.2-9.8
std. dev.	*0.2*	*1.1*	*0.6*	*3.0*	*0.4*	*0.4*	

Table 12. continued

DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE(P<0.05)
			PERCENT METHANE IN HEADSPACE				
35	6.8	13.0	9.8	7.0	2.3	2.4	2.9-10.6
std. dev.	*0.1*	*1.5*	*0.4*	*3.5*	*0.5*	*0.4*	
42	7.5	15.3	10.9	9.2	2.9	2.6	4.2-10.7
std. dev.	*0.2*	*0.8*	*0.5*	*2.9*	*0.5*	*0.5*	
51	7.9	16.8	11.3	12.4	4.8	2.8	7.0-8.8
std. dev.	*0.1*	*0.5*	*0.5*	*0.4*	*0.2*	*0.5*	
59	8.2	17.5	11.9	13.3	7.2	3.0	7.3-9.2
std. dev.							
70	8.0	16.3	11.2	12.6	12.0	3.0	5.7-10.3
std. dev.	*0.2*	*0.3*	*0.6*	*0.1*	*2.0*	*0.5*	
82	8.0	16.1	11.2	12.8	14.3	3.0	6.8-9.1
std. dev.	*0.3*	*0.3*	*0.7*	*0.1*	*0.4*	*0.7*	
91	7.9	16.4	11.4	13.0	13.1	3.5	5.3-10.6
std. dev.	*0.2*	*0.1*	*1.0*	*0.1*	*1.7*	*1.4*	

Table 13. Nitrogen stripped sample B culture dilutions (Experiment 2B1).

	PERCENT METHANE IN HEADSPACE						
DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE(P<0.05)
1	1.7	1.7	1.5	2.0	1.3	0.9	
std. dev.	*0.4*	*0.4*	*1.2*	*0.4*	*0.6*	*0.6*	
2	2.5	2.6	1.9	3.3	1.7	1.2	
std. dev.	*0.3*	*0.3*	*1.5*	*0.8*	*1.0*	*0.7*	
3	3.2	3.2	2.8	3.9	2.0	1.4	0.8-5.5
std. dev.	*0.4*	*0.4*	*1.2*	*0.9*	*1.2*	*0.9*	
4	3.7	3.7	3.7	4.0	2.1	1.5	1.6-5.8
std. dev.	*0.4*	*0.3*	*0.8*	*0.9*	*1.1*	*0.9*	
5	4.1	4.0	4.1	4.3	2.0	1.5	2.0-6.2
std. dev.	*0.4*	*0.3*	*0.6*	*1.0*	*1.2*	*0.9*	
7	4.5	4.4	4.3	4.4	2.3	1.6	2.5-6.5
std. dev.	*0.3*	*0.3*	*0.5*	*1.0*	*1.2*	*0.9*	
9	4.7	4.7	4.4	4.7	2.2	1.5	2.8-6.6
std. dev.	*0.4*	*0.3*	*0.3*	*1.1*	*1.1*	*0.7*	
11	4.9	4.7	4.7	5.0	2.2	1.1	2.7-7.1
std. dev.	*0.4*	*0.1*	*0.5*	*1.2*	*1.2*	*0.9*	
15	5.2	5.2	5.4	5.7	2.3	1.6	3.0-7.5
std. dev.	*0.4*	*0.3*	*0.7*	*1.2*	*1.2*	*0.8*	
20	5.5	5.9	5.7	6.3	2.4	1.6	3.3-7.8
std. dev.	*0.3*	*0.4*	*0.8*	*0.9*	*1.4*	*0.8*	
25	5.9	7.1	6.4	6.8	2.8	1.8	3.6-8.3
std. dev.	*0.2*	*0.6*	*0.6*	*0.7*	*1.7*	*0.9*	
30	6.5	9.4	6.8	7.7	3.6	2.2	3.5-9.4
std. dev.	*0.2*	*1.1*	*0.5*	*0.4*	*2.4*	*1.1*	

Table 13. continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE(P<0.05)
35	6.8	13.0	7.3	8.1	4.4	2.9	3.0-10.5
std. dev.	*0.1*	*1.5*	*0.7*	*0.3*	*2.8*	*1.9*	
42	7.5	15.3	8.1	9.0	6.3	4.8	2.5-12.5
std. dev.	*0.2*	*0.8*	*0.8*	*0.3*	*3.5*	*2.9*	
51	7.9	16.8	8.6	9.5	7.9	7.0	4.3-11.6
std. dev.	*0.1*	*0.5*	*0.9*	*0.2*	*2.3*	*2.3*	
59	8.2	17.5	8.9	9.9	9.7	8.7	5.5-10.9
std. dev.	*0.2*	*0.2*	*0.8*	*0.3*	*1.2*	*2.0*	
70	8.0	16.3	8.8	9.5	9.8	9.3	5.4-10.6
std. dev.	*0.2*	*0.3*	*0.8*	*0.4*	*0.7*	*2.1*	
82	8.0	16.1	9.0	9.6	9.9	10.2	6.6-9.4
std. dev.	*0.3*	*0.3*	*0.7*	*0.4*	*0.4*	*0.8*	
91	7.9	16.4	8.7	8.7	10.0	10.5	5.1-10.8
std. dev.	*0.2*	*0.1*	*1.3*	*2.0*	*0.4*	*0.9*	

Table 14. Nitrogen stripped sample C culture dilutions (Experiment 2C1).

	PERCENT METHANE IN HEADSPACE						
DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.7	1.7	1.0	0.8	0.5	0.5	
std. dev.	*0.4*	*0.4*	*0.3*	*0.3*	*0.1*	*0.1*	
2	2.5	2.6	2.1	1.7	1.0	0.6	0.7-4.3
std. dev.	*0.3*	*0.3*	*0.9*	*1.4*	*0.4*	*0.1*	
3	3.2	3.2	3.1	2.4	1.7	0.8	1.8-4.5
std. dev.	*0.4*	*0.4*	*0.6*	*1.3*	*0.7*	*0.2*	
4	3.7	3.7	3.3	3.0	2.0	0.7	2.0-5.5
std. dev.	*0.4*	*0.3*	*0.3*	*1.1*	*1.0*	*0.2*	
5	4.1	4.0	3.8	3.5	2.7	0.8	2.2-6.0
std. dev.	*0.4*	*0.3*	*0.4*	*1.0*	*1.3*	*0.5*	
7	4.5	4.4	4.1	4.0	3.3	1.1	2.7-6.3
std. dev.	*0.3*	*0.3*	*0.3*	*0.6*	*1.5*	*0.3*	
9	4.7	4.7	4.1	4.3	3.1	1.0	2.7-6.7
std. dev.	*0.4*	*0.3*	*0.3*	*0.4*	*1.7*	*0.3*	
11	4.9	4.7	4.1	4.5	3.5	0.9	3.0-6.8
std. dev.	*0.4*	*0.1*	*0.5*	*0.2*	*1.6*	*0.3*	
15	5.2	5.2	4.5	4.8	3.9	1.1	3.2-7.3
std. dev.	*0.4*	*0.3*	*0.3*	*0.4*	*1.8*	*0.3*	
20	5.5	5.9	4.7	4.9	4.1	1.2	3.4-7.7
std. dev.	*0.3*	*0.4*	*0.4*	*1.8*	*1.8*	*0.3*	
25	5.9	7.1	5.1	5.3	4.3	1.2	3.8-8.1
std. dev.	*0.2*	*0.6*	*0.2*	*0.5*	*1.9*	*0.3*	
30	6.5	9.4	5.7	5.6	4.5	1.3	4.1-8.4
std. dev.	*0.2*	*1.1*	*0.1*	*0.5*	*1.9*	*0.3*	

Table 14. continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
35	6.8	13.0	6.8	6.2	4.9	1.2	4.4-9.2
std. dev.	*0.1*	*1.5*	*0.2*	*0.5*	*2.1*	*0.3*	
42	7.5	15.3	7.5	7.3	5.5	1.2	5.0-10.0
std. dev.	*0.2*	*0.8*	*0.2*	*0.6*	*2.2*	*0.4*	
51	7.9	16.8	8.0	8.4	6.0	1.5	5.3-10.5
std. dev.	*0.1*	*0.5*	*0.2*	*0.7*	*2.2*	*0.4*	
59	8.2	17.5	8.3	8.9	6.5	1.9	5.7-10.7
std. dev.	0.2		0.2	0.7	2.1	0.4	
70	8.0	16.3	7.5	8.3	6.6	3.8	5.6-10.3
std. dev.	*0.2*	*0.3*	*0.8*	*0.8*	*1.5*	*1.0*	
82	8.0	16.1	8.0	8.4	7.3	5.1	5.9-10.1
std. dev.	*0.3*	*0.3*	*0.3*	*0.6*	*1.8*	*0.3*	
91	7.9	16.4	7.9	8.3	7.5	5.3	5.8-10.1
std. dev.	*0.2*	*0.4*	*0.3*	*0.6*	*1.8*	*0.4*	

Table 15. Culture dilutions (50% sample concentration) for: Nitrogen stripped sample A, sample A inorganics, and sample A pretreated by batch adsorption with activated carbon (10 g activated carbon/L); Experiments 2A2 and 2A3.

	PERCENT METHANE IN HEADSPACE							
DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIGNIF. RANGE (P<0.05)
1	1.7	1.7	0.4	0.5	0.3	0.5	0.5	
std. dev.	*0.4*	*0.4*	*0.2*	*0.1*	*0.2*	*0.2*	*0.2*	
2	2.6	2.5	0.5	0.7	0.4	0.6	0.6	1.9-3.3
std. dev.	*0.3*	*0.3*	*0.3*	*0.2*	*0.4*	*0.3*	*0.3*	
3	3.2	3.2	0.6	0.8	0.4	0.7	0.8	2.1-4.2
std. dev.	*0.4*	*0.4*	*0.5*	*0.2*	*0.6*	*0.4*	*0.4*	
4	3.7	3.7	0.7	0.9	0.6	0.8	0.8	2.5-4.9
std. dev.	*0.3*	*0.4*	*0.5*	*0.3*	*0.7*	*0.6*	*0.4*	
5	4.0	4.1	0.9	1.0	0.7	0.9	0.9	2.4-5.6
std. dev.	*0.3*	*0.4*	*0.9*	*0.4*	*1.0*	*0.7*	*0.5*	
7	4.4	4.5	1.1	1.2	0.9	1.1	1.2	2.7-6.1
std. dev.	*0.3*	*0.3*	*0.9*	*0.5*	*1.1*	*0.7*	*0.6*	
9	4.7	4.7	1.4	1.3	1.1	1.3	1.4	2.9-6.4
std. dev.	*0.3*	*0.4*	*0.8*	*0.5*	*1.0*	*1.0*	*0.6*	
11	4.7	4.9	1.5	1.5	0.9	1.5	1.8	3.1-6.2
std. dev.	*0.1*	*0.4*	*0.7*	*0.4*	*0.7*	*1.0*	*0.6*	
15	5.2	5.2	1.7	1.6	1.6	1.7	2.1	4.0-6.5
std. dev.	*0.3*	*0.4*	*0.5*	*0.4*	*0.8*	*0.8*	*0.3*	
20	5.9	5.5	2.0	1.7	1.8	1.9	1.9	4.8-7.0
std. dev.	*0.4*	*0.3*	*0.3*	*0.3*	*0.6*	*0.5*	*0.6*	
25	7.1	5.9	1.8	1.8	1.9	2.3	2.3	6.2-8.14
std. dev.	*0.6*	*0.2*	*0.3*	*0.3*	*0.6*	*0.2*	*0.3*	
30	9.4	6.3	2.4	1.9	2.1	2.5	2.4	8.4-11.1
std. dev.	*1.1*	*0.2*	*0.4*	*0.3*	*0.6*	*0.3*	*0.3*	

Table 15. continued

PERCENT METHANE IN HEADSPACE

DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIGNIF. RANGE (P<0.05)
35	13.0	6.8	2.4	1.8	2.0	2.6	2.4	11.3-14.7
std. dev.	*1.5*	*0.1*	*0.4*	*0.3*	*0.7*	*0.4*	*0.3*	
42	15.3	7.5	2.6	1.9	2.2	2.8	2.7	13.9-16.7
std. dev.	*0.8*	*0.2*	*0.5*	*0.3*	*0.8*	*0.6*	*0.3*	
51	16.8	7.9	2.8	2.2	2.5	3.1	3.6	15.0-18.6
std. dev.	*0.5*	*0.1*	*0.5*	*0.2*	*0.8*	*0.8*	*1.3*	
59	17.5	8.2	3.0	2.4	2.7	3.6	4.6	14.7-20.3
std. dev.	*0.2*	*0.2*	*0.5*	*0.2*	*1.0*	*0.9*	*2.6*	
70	16.3	8.0	3.0	2.8	3.0	4.7	5.1	12.8-19.6
std. dev.	*0.3*	*0.2*	*0.6*	*0.5*	*1.1*	*1.7*	*2.9*	
82	16.1	8.0	3.0	4.2	3.9	5.1	8.6	9.3-23.0
std. dev.	*0.3*	*0.3*	*0.7*	*1.9*	*2.1*	*2.3*	*6.4*	
91	16.4	7.9	3.5	4.1	5.6	5.5	9.2	8.2-24.6
std. dev.	*0.4*	*0.2*	*1.4*	*2.3*	*3.5*	*3.0*	*7.0*	

Table 16. Culture dilutions (50% sample concentration) for: Nitrogen stripped sample B, sample B inorganics, and sample B pretreated by batch adsorption with activated carbon (10 g activated carbon/L); (Experiments 282 and 283).

	PERCENT METHANE IN HEADSPACE							
DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIGNIF. RANGE (P≤0.05)
1	1.7	1.7	0.9	1.1	0.9	0.8	0.9	
std. dev.	*0.4*	*0.4*	*0.6*	*0.5*	*0.3*	*0.5*	*0.5*	
2	2.6	2.5	0.5	0.7	0.4	0.6	0.6	1.9-3.3
std. dev.	*0.3*	*0.3*	*0.3*	*0.2*	*0.4*	*0.2*	*0.3*	
3	3.2	3.2	1.4	1.9	1.2	1.3	1.5	1.3-5.0
std. dev.	*0.4*	*0.4*	*0.9*	*0.9*	*0.4*	*0.8*	*1.1*	
4	3.7	3.7	1.5	1.9	1.2	1.3	1.5	1.8-5.6
std. dev.	*0.3*	*0.4*	*0.9*	*0.9*	*0.3*	*0.7*	*1.3*	
5	4.0	4.1	1.5	2.0	1.4	1.4	1.7	2.1-5.9
std. dev.	*0.3*	*0.4*	*0.9*	*0.9*	*0.5*	*0.8*	*1.3*	
7	4.4	4.5	1.6	2.0	1.5	1.5	1.8	2.6-6.2
std. dev.	*0.3*	*0.3*	*0.9*	*0.9*	*0.4*	*0.8*	*1.2*	
9	4.7	4.7	1.5	2.0	1.5	1.5	1.8	2.9-6.4
std. dev.	*0.3*	*0.4*	*0.7*	*0.9*	*0.4*	*0.7*	*1.2*	
11	4.7	4.8	1.4	1.9	1.5	1.5	1.9	2.7-6.6
std. dev.	*0.1*	*0.4*	*0.9*	*1.0*	*0.5*	*0.7*	*1.4*	
15	5.2	5.2	1.6	2.0	1.5	1.7	2.2	3.1-7.4
std. dev.	*0.3*	*0.4*	*0.8*	*0.9*	*0.5*	*0.7*	*1.8*	
20	5.9	5.5	1.6	1.4	1.4	2.0	2.6	3.2-8.6
std. dev.	*0.4*	*0.3*	*0.8*	*0.5*	*0.4*	*0.9*	*2.5*	
25	7.1	5.9	1.8	1.6	1.6	2.9	2.9	3.9-10.4
std. dev.	*0.6*	*0.2*	*0.9*	*0.6*	*0.5*	*1.6*	*2.8*	
30	9.4	6.3	2.2	1.9	1.7	4.1	3.0	5.6-13.2
std. dev.	*1.1*	*0.2*	*1.1*	*0.9*	*0.5*	*2.2*	*2.8*	

Table 16. continued

DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIGNIF. RANGE (P<0.05)
	PERCENT METHANE IN HEADSPACE							
35	13.0	6.8	2.9	2.3	1.6	5.2	3.4	8.6-17.4
std. dev.	*1.5*	*0.1*	*1.9*	*1.5*	*0.6*	*1.7*	*3.3*	
42	15.3	7.5	4.8	3.6	2.1	6.4	4.8	9.3-21.3
std. dev.	*0.8*	*0.2*	*2.9*	*3.0*	*0.8*	*1.3*	*4.6*	
51	16.8	7.9	7.0	4.9	4.1	7.5	8.7	8.8-24.8
std. dev.	*0.5*	*0.1*	*2.3*	*3.3*	*1.6*	*1.6*	*7.3*	
59	17.5	8.2	8.7	5.5	6.7	9.2	10.2	9.6-25.3
std. dev.	*0.2*	*0.2*	*2.0*	*3.7*	*1.1*	*2.9*	*6.7*	
70	16.3	8.0	9.3	6.6	7.2	13.7	10.7	8.6-23.9
std. dev.	*0.3*	*0.2*	*2.1*	*4.3*	*0.4*	*4.0*	*5.3*	
82	16.1	8.0	10.2	10.6	11.5	16.5	13.0	8.3-24.0
std. dev.	*0.3*	*0.3*	*0.8*	*5.9*	*2.6*	*0.7*	*5.4*	
91	16.4	7.9	10.5	12.7	14.7	16.9	15.0	9.8-23.0
std. dev.	*0.4*	*0.2*	*0.9*	*6.5*	*0.6*	*0.5*	*2.5*	

Table 17. Culture dilutions (50% sample concentration) for: Nitrogen stripped sample C, sample C inorganics, and sample C pretreated by batch adsorption with activated carbon (10 g activated carbon/L); (Experiments 2C2 and 2C3).

	PERCENT METHANE IN HEADSPACE							
DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIG. RANGE (P<0.05)
1	1.7	1.7	0.5	0.4	0.4	0.4	0.3	
std. dev.	*0.4*	*0.4*	*0.1*	*0.2*	*0.2*	*0.2*	*0.2*	
2	2.6	2.5	0.6	0.5	0.4	0.4	0.4	2.0-3.2
std. dev.	*0.3*	*0.3*	*0.1*	*0.3*	*0.3*	*0.2*	*0.2*	
3	3.2	3.2	0.8	0.7	0.5	0.5	0.6	2.4-3.9
std. dev.	*0.4*	*0.4*	*0.2*	*0.3*	*0.4*	*0.3*	*0.2*	
4	3.7	3.7	0.7	0.8	0.5	0.5	0.6	2.9-4.6
std. dev.	*0.3*	*0.4*	*0.2*	*0.4*	*0.4*	*0.4*	*0.4*	
5	4.0	4.1	0.8	0.7	0.6	0.4	0.8	3.0-5.0
std. dev.	*0.3*	*0.4*	*0.5*	*0.5*	*0.5*	*0.5*	*0.2*	
7	4.4	4.5	1.1	0.9	0.8	0.7	1.1	3.5-5.3
std. dev.	*0.3*	*0.3*	*0.3*	*0.4*	*0.6*	*0.4*	*0.2*	
9	4.7	4.7	1.0	0.9	0.8	0.7	0.9	3.6-5.7
std. dev.	*0.3*	*0.4*	*0.3*	*0.4*	*0.6*	*0.4*	*0.6*	
11	4.7	4.9	0.9	0.9	0.7	0.7	1.1	3.8-5.5
std. dev.	*0.1*	*0.4*	*0.3*	*0.4*	*0.6*	*0.3*	*0.2*	
15	5.2	5.2	1.1	0.9	0.8	0.8	1.3	4.4-6.1
std. dev.	*0.3*	*0.4*	*0.3*	*0.4*	*0.5*	*0.3*	*0.2*	
20	5.9	5.5	1.2	1.0	0.9	0.9	0.9	4.9-6.9
std. dev.	*0.4*	*0.3*	*0.3*	*0.4*	*0.5*	*0.2*	*0.6*	
25	7.1	5.9	1.8	1.6	1.6	1.1	1.5	6.2-8.1
std. dev.	*0.6*	*0.2*	*0.3*	*0.4*	*0.5*	*0.2*	*0.2*	
30	9.4	6.3	1.3	1.2	1.0	1.3	1.7	8.1-10.7
std. dev.	*1.1*	*0.2*	*0.3*	*0.3*	*0.6*	*0.3*	*0.3*	

Table 17. continued

PERCENT METHANE IN HEADSPACE

DAY	PHENOL CONTROL	CONTROL	50% SAMPLE	INORG. PHASE	1 g/l A.C.	4 g/l A.C.	10 g/l A.C.	NON-SIG. RANGE (P<0.05)
35	13.0	6.8	1.2	1.1	0.9	1.4	1.8	11.4-14.6
std. dev.	*1.5*	*0.1*	*0.3*	*0.3*	*0.6*	*0.4*	*0.2*	
42	15.3	7.5	1.2	1.2	0.9	1.8	2.1	14.0-16.6
std. dev.	*0.8*	*0.2*	*0.3*	*0.2*	*0.8*	*0.8*	*0.1*	
51	16.8	7.9	1.5	1.6	1.2	2.9	2.4	15.5-18.1
std. dev.	*0.5*	*0.1*	*0.4*	*0.1*	*0.9*	*0.7*	*0.4*	
59	17.5	8.2	1.9	2.0	1.5	3.9	5.0	16.2-18.7
std. dev.	*0.2*	*0.2*	*0.4*	*0.5*	*1.1*	*0.2*	*0.1*	
70	16.3	8.0	3.8	2.3	2.2	4.2	4.5	14.4-18.1
std. dev.	*0.3*	*0.2*	*1.0*	*0.4*	*1.5*	*0.5*	*0.4*	
82	16.1	8.0	5.1	3.4	1.7	4.2	4.7	13.8-18.5
std. dev.	*0.3*	*0.3*	*0.3*	*0.5*	*2.3*	*0.8*	*0.5*	
91	16.4	7.9	5.3	3.8	2.9	4.1		13.7-19.1
std. dev.	*0.4*	*0.2*	*0.4*	*0.8*	*2.4*	*0.7*		

Table 18. Sample A (50% sample concentration) culture dilution enriched with acetate (Experiment 2A4).

DAY	PERCENT METHANE IN HEADSPACE				
	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	2.5	1.7	0.4	0.4	
std. dev.	*1.9*	*0.4*	*0.2*	*0.1*	
2	8.0	2.5	0.5	0.5	3.9-12.1
std. dev.	*3.4*	*0.3*	*0.3*	*0.1*	
3	11.9	3.2	0.6	0.6	9.2-14.6
std. dev.	*2.2*	*0.4*	*0.5*	*0.2*	
4	13.0	3.7	0.7	0.7	12.1-14.0
std. dev.	*0.3*	*0.4*	*0.5*	*0.3*	
5	14.1	4.1	0.9	0.7	12.9-15.3
std. dev.	*0.3*	*0.4*	*0.9*	*0.1*	
7	14.0	4.5	1.1	0.9	12.8-15.3
std. dev.	*0.2*	*0.3*	*0.9*	*0.3*	
9	14.1	4.7	1.4	1.2	13.0-15.3
std. dev.	*0.3*	*0.4*	*0.8*	*0.1*	
11	14.6	4.9	1.5	1.2	13.2-16.0
std. dev.	*0.2*	*0.4*	*0.7*	*0.9*	
15	14.6	5.2	1.7	2.9	13.6-15.7
std. dev.	*0.1*	*0.4*	*0.5*	*0.7*	
20	14.8	5.5	2.0	3.2	13.8-15.5
std. dev.		*0.3*	*0.3*	*0.8*	
25	15.1	5.9	2.2	3.9	14.1-16.1
std. dev.	*0.1*	*0.3*	*0.3*	*0.6*	
30	15.4	6.3	2.4	5.8	14.8-17.1
std. dev.	*0.1*	*0.2*	*0.4*	*0.8*	

Table 18. continued

PERCENT METHANE IN HEADSPACE

DAY	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
35	16.7	6.8	2.4	8.8	15.5-18.0
std. dev.	*0.1*	*0.1*	*0.4*	*0.9*	
42	18.2	7.5	2.6	12.5	16.7-19.7
std. dev.	*0.1*	*0.2*	*0.5*	*1.2*	
51	18.2	7.9	2.8	15.9	16.8-19.7
std. dev.	*0.1*	*0.1*	*0.5*	*1.1*	
59	19.0	8.2	3.0	18.1	18.2-19.8
std. dev.	*0.1*	*0.2*	*0.5*	*0.4*	
70	17.5	8.0	3.0	17.3	16.9-18.8
std. dev.	*0.1*	*0.2*	*0.5*	*0.1*	
82	17.6	8.0	3.0	17.3	16.8-18.5
std. dev.	*0.2*	*0.3*	*0.7*	*0.1*	
91	17.5	7.9	3.5	17.5	15.0-20.1
std. dev.	*0.4*	*0.2*	*1.4*	*1.5*	

Table 19. Sample B (50% sample concentration) culture dilution enriched with acetate (Experiment 284).

DAY	PERCENT METHANE IN HEADSPACE				
	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	2.5	1.7	0.9	0.5	
std. dev.	*1.9*	*0.4*	*0.6*	*0.2*	
2	8.0	2.5	0.5	0.5	3.9-12.1
std. dev.	*3.4*	*0.3*	*0.3*	*0.1*	
3	11.9	3.2	1.4	0.6	9.0-14.8
std. dev.	*2.2*	*0.4*	*0.9*	*0.4*	
4	13.0	3.7	1.5	0.7	11.7-14.4
std. dev.	*0.3*	*0.4*	*0.9*	*0.4*	
5	14.1	4.1	1.5	0.7	12.8-15.4
std. dev.	*0.3*	*0.4*	*0.9*	*0.4*	
7	14.0	4.5	1.6	0.8	12.8-15.3
std. dev.	*0.2*	*0.3*	*0.9*	*0.4*	
9	14.1	4.7	1.5	0.4	13.0-15.2
std. dev.	*0.3*	*0.4*	*0.7*	*0.4*	
11	14.6	4.9	1.4	0.9	13.4-15.8
std. dev.	*0.2*	*0.4*	*0.9*	*0.3*	
15	14.6	5.2	1.6	1.0	13.5-15.8
std. dev.	*0.1*	*0.4*	*0.8*	*0.2*	
20	14.8	5.5	1.6	2.4	13.0-16.7
std. dev.		*0.3*	*0.8*	*1.3*	
25	15.1	5.9	1.8	4.8	10.3-20.0
std. dev.	*0.1*	*0.3*	*0.9*	*3.9*	
30	15.4	6.3	2.2	7.6	8.4-22.4
std. dev.	*0.1*	*0.2*	*1.1*	*5.7*	

Table 19. continued

PERCENT METHANE IN HEADSPACE

DAY	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
35	16.7	6.8	2.9	10.1	10.9-22.6
std. dev.	*0.1*	*0.1*	*1.9*	*4.5*	
42	18.2	7.5	4.8	16.0	14.4-21.9
std. dev.	*0.1*	*0.2*	*2.9*	*1.1*	
51	18.2	7.9	7.0	17.7	15.2-21.2
std. dev.	*0.1*	*0.1*	*2.3*	*1.0*	
59	19.0	8.2	8.7	18.2	16.3-21.7
std. dev.	*0.1*	*0.2*	*2.0*	*1.1*	
70	17.5	8.0	9.3	17.0	14.7-20.4
std. dev.	*0.1*	*0.2*	*2.1*	*1.1*	
82	17.6	8.0	10.2	17.4	16.1-19.1
std. dev.	*0.2*	*0.3*	*0.8*	*0.9*	
91	17.5	7.9	10.5	17.5	16.0-19.0
std. dev.	*0.4*	*0.2*	*0.9*	*0.8*	

Table 20. Sample A (50% sample concentration) culture dilution enriched with acetate (Experiment 2C4).

	PERCENT METHANE IN HEADSPACE				
DAY	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	2.5	1.7	0.5	0.4	
std. dev.	*1.9*	*0.4*	*0.1*	*0.1*	
2	8.0	2.5	0.6	0.4	
std. dev.	*3.4*	*0.3*	*0.1*	*0.2*	
3	11.9	3.2	0.8	0.5	9.2-14.6
std. dev.	*2.2*	*0.4*	*0.2*	*0.3*	
4	13.0	3.7	0.7	0.6	12.3-13.8
std. dev.	*0.3*	*0.4*	*0.2*	*0.3*	
5	14.1	4.1	0.8	0.7	13.2-15.5
std. dev.	*0.3*	*0.4*	*0.5*	*0.3*	
7	14.0	4.5	1.1	1.0	13.4-14.7
std. dev.	*0.2*	*0.3*	*0.3*	*0.2*	
9	14.1	4.7	1.0	1.0	13.4-14.8
std. dev.	*0.3*	*0.4*	*0.3*	*0.2*	
11	14.6	4.9	0.9	1.1	13.9-15.3
std. dev.	*0.2*	*0.4*	*0.3*	*0.2*	
15	14.6	5.2	1.1	1.2	14.0-15.3
std. dev.	*0.1*	*0.4*	*0.3*	*0.2*	
20	14.8	5.5	1.2	2.2	14.2-15.4
std. dev.		*0.3*	*0.3*	*0.3*	
25	15.1	5.9	1.2	3.9	13.7-16.5
std. dev.	*0.1*	*0.3*	*0.3*	*1.1*	
30	15.4	6.3	1.3	7.6	14.0-16.8
std. dev.	*0.1*	*0.2*	*0.3*	*1.1*	

Table 20. continued

PERCENT METHANE IN HEADSPACE

DAY	ACETATE CONTROL	CONTROL	50% SAMPLE	ACETATE SAMPLE	NON-SIGNIF. RANGE (P<0.05)
35	16.7	6.8	1.2	10.3	14.8-18.7
std. dev.	*0.1*	*0.1*	*0.3*	*1.6*	
42	18.2	7.5	1.2	12.8	16.3-20.0
std. dev.	*0.1*	*0.2*	*0.4*	*1.5*	
51	18.2	7.9	1.5	14.7	16.7-19.7
std. dev.	*0.1*	*0.1*	*0.4*	*1.2*	
59	19.0	8.2	1.9	16.4	17.4-20.6
std. dev.	*0.1*	*0.2*	*0.4*	*1.3*	
70	17.5	8.0	3.8	16.7	15.7-19.3
std. dev.	*0.1*	*0.2*	*1.0*	*1.1*	
82	17.6	8.0	5.1	15.9	16.0-19.3
std. dev.	*0.2*	*0.3*	*0.3*	*1.3*	
91	17.5	7.9	5.3	16.1	15.6-19.4
std. dev.	*0.4*	*0.2*	*0.4*	*1.5*	

Table 21. Culture dilutions for nitrogen stripped sample A and batch adsorption with activated carbon pretreated sample A (Experiments 3A1 and 3A2).

	PERCENT METHANE IN HEADSPACE								
DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	40% A.C. SAMPLE	50% A.C. SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	3.4	3.2	1.1	0.6	0.6	0.6	1.1	0.6	1.1-2.5
std. dev.	*0.3*	*0.4*	*0.1*	*0.1*	*0.1*	*0.1*	*0.2*	*0.1*	
3	5.6	5.6	3.5	1.2	1.1	1.0	2.7	1.1	4.9-6.3
std. dev.	*0.3*	*0.4*	*0.4*	*0.1*	*0.1*	*0.2*	*0.5*	*0.1*	
5	8.3	8.4	7.3	2.0	1.8	1.4	5.7	1.8	6.8-9.7
std. dev.	*0.2*	*0.5*	*0.8*	*0.2*	*0.2*	*0.4*	*1.3*	*0.2*	
7	8.0	8.1	7.6	2.5	2.5	1.9	6.5	2.2	6.6-9.3
std. dev.	*0.1*	*0.4*	*0.7*	*0.2*	*0.1*	*0.7*	*1.2*	*0.1*	
10	8.5	9.0	8.2	2.8	2.8	2.6	7.5	2.3	7.5-9.5
std. dev.	*0.1*	*0.3*	*0.7*	*0.1*	*0.1*	*0.4*	*0.7*	*0.1*	
14	9.6	12.0	10.7	3.3	3.2	3.1	8.6	2.3	8.4-10.8
std. dev.	*0.2*	*0.7*	*0.4*	*0.3*	*0.1*	*0.3*	*0.7*	*0.7*	
21	10.8	15.8	12.9	4.0	3.5	3.3	10.7	3.1	9.6-12.0
std. dev.	*0.1*	*0.5*	*0.6*	*0.2*	*0.1*	*0.3*	*1.0*	*0.2*	
26	11.6	16.7	13.5	4.7	3.8	3.5	13.5	3.6	10.4-12.8
std. dev.	*0.3*	*0.4*	*0.5*	*0.4*	*0.2*	*0.3*	*1.0*	*0.3*	
35	12.0	17.1	13.8	6.3	4.1	3.5	15.0	4.7	9.9-14.2
std. dev.	*0.3*	*0.4*	*0.4*	*1.0*	*0.5*	*0.3*	*1.9*	*0.7*	
43	12.4	17.2	14.1	8.7	4.9	3.7	15.1	7.1	9.8-14.9
std. dev.	*0.3*	*0.4*	*0.6*	*1.4*	*1.0*	*0.2*	*1.6*	*1.5*	
52	12.9	17.5	14.4	11.9	5.9	4.0	15.6	11.1	9.4-16.4
std. dev.	*0.2*	*0.6*	*0.8*	*1.8*	*1.0*	*0.3*	*1.8*	*2.8*	
60	13.5	18.4	15.1	15.3	7.6	4.3	16.3	14.1	10.2-16.7
std. dev.	*0.3*	*0.4*	*0.6*	*2.0*	*1.7*	*0.3*	*1.7*	*1.9*	

Table 21. continued

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	40% A.C. SAMPLE	50% A.C. SAMPLE	NON-SIGNIF. RANGE (P<0.05)
74	13.7	18.5	15.5	17.1	10.6	4.5	16.6	15.1	10.9-16.5
std. dev.	*0.3*	*0.6*	*0.4*	*0.5*	*2.3*	*0.4*	*1.7*	*1.0*	
82	13.6	18.3	15.4	16.9	12.5	4.8	16.6	14.4	9.7-17.1
std. dev.	*0.3*	*0.5*	*0.6*	*0.7*	*2.8*	*1.9*	*2.0*	*1.8*	
89	14.2	18.8	16.0	16.9	15.8	6.8	16.8	15.8	9.1-19.2
std. dev.	*0.4*	*0.5*	*0.6*	*1.1*	*3.6*	*3.6*	*2.1*	*0.4*	
96	13.4	18.1	15.4	17.2	16.5	7.0	16.8	15.2	8.7-18.1
std. dev.	*0.5*	*0.6*	*0.6*	*0.8*	*2.1*	*4.3*	*2.0*	*0.3*	

Table 22. Nitrogen stripped sample D culture dilutions (Experiment 3D1).

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
	PERCENT METHANE IN HEADSPACE							
1	3.4	3.2	0.8	0.6	0.4	0.4	0.4	3.0-3,9
std. dev.	*0.3*	*0.4*	*0.1*	*0.1*	*0.1*	*0.1*	*0.1*	
3	5.6	5.6	1.4	1.1	0.7	0.8	0.7	5.1-6.1
std. dev.	*0.3*	*0.4*	*0.1*	*0.1*	*0.1*	*0.2*	*0.2*	
5	8.3	8.4	2.1	1.7	1.1	1.0	0.8	7.7-8.9
std. dev.	*0.2*	*0.5*	*0.1*	*0.1*	*0.1*	*0.3*	*0.2*	
7	8.0	8.1	2.2	1.9	1.4	1.2	1.0	7.5-8.5
std. dev.	*0.1*	*0.4*	*0.1*	*0.1*	*0.1*	*0.2*	*0.2*	
10	8.5	9.0	2.4	2.0	1.7	1.4	1.1	8.1-8.9
std. dev.	*0.1*	*0.3*	*0.1*	*0.1*	*0.1*	*0.1*	*0.2*	
14	9.6	12.0	3.2	2.4	1.8	1.8	1.5	8.8-10.5
std. dev.	*0.2*	*0.7*	*0.2*	*0.1*	*0.5*	*0.2*	*0.1*	
21	10.8	15.8	4.5	2.7	2.4	2.1	1.7	10.1-11.5
std. dev.	*0.1*	*0.5*	*0.5*	*0.1*	*0.2*	*0.1*	*0.1*	
26	11.6	16.7	6.9	3.1	2.7	2.3	1.9	10.2-13.1
std. dev.	*0.3*	*0.4*	*1.4*	*0.1*	*0.1*	*0.1*	*0.1*	
35	12.0	17.1	11.6	3.3	3.0	2.1	1.7	11.4-12.7
std. dev.	*0.3*	*0.4*	*0.4*	*0.2*	*0.1*	*0.1*	*0.1*	
43	12.4	17.2	12.2	3.8	4.9	2.4	2.0	11.4-13.4
std. dev.	*0.3*	*0.4*	*0.6*	*0.1*	*0.7*	*0.1*	*0.2*	
52	12.9	17.5	12.5	4.1	7.1	2.5	2.0	11.2-14.6
std. dev.	*0.2*	*0.6*	*0.7*	*0.1*	*1.6*	*0.1*	*2.0*	
60	13.5	18.4	13.5	4.91	9.2	2.8	2.4	12.4-14.5
std. dev.	*0.3*	*0.4*	*0.6*	*0.5*	*0.7*	*0.1*	*0.1*	

PERCENT METHANE IN HEADSPACE

DAY	CONTROL	PHENOL CONTROL	10% SAMPLE	20% SAMPLE	30% SAMPLE	40% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
74	13.7	18.5	13.0	11.8	12.7	2.9	2.3	11.1-16.2
std. dev.	*0.3*	*0.6*	*2.1*	*0.5*	*1.5*	*0.1*	*0.4*	
82	13.6	18.3	13.8	13.3	15.0	2.9	2.2	12.1-15.1
std. dev.	*0.3*	*0.5*	*0.6*	*1.2*	*0.4*	*0.1*	*0.5*	
89	14.2	18.8	14.1	15.5	15.8	3.0	2.5	12.8-15.6
std. dev.	*0.4*	*0.5*	*0.6*	*1.0*	*0.6*	*0.1*	*0.2*	
96	13.4	18.1	13.7	15.4	15.2	2.9	2.5	12.1-14.7
std. dev.	*0.5*	*0.6*	*0.6*	*0.7*	*0.7*	*0.1*	*0.1*	

Table 23. Culture dilutions (50% sample concentrations) for sample A organic and inorganic components (Experiment 5A).

DAY	PERCENT METHANE IN HEADSPACE					
	CONTROL	PHENOL CONTROL	50% SAMPLE	ORG. PHASE	INORG. PHASE	NON-SIGNIF. RANGE(P<0.05)
3	5.4	5.3	1.5	5.0	1.7	4.8-6.0
std. dev.	*0.4*	*0.1*	*0.1*	*0.3*	*0.1*	
7	7.1	7.3	3.1	7.2	3.1	6.4-7.7
std. dev.	*0.5*	*0.1*	*0.2*	*0.3*	*0.1*	
10	8.3	8.1	3.8	8.7	4.0	6.8-9.7
std. dev.	*0.5*	*1.2*	*0.3*	*0.3*	*0.1*	
16	9.1	12.8	4.4	12.0	5.4	7.3-10.9
std. dev.	*0.3*	*1.1*	*0.4*	*1.1*	*0.2*	
23	10.4	15.1	4.6	15.9	8.3	7.2-13.5
std. dev.	*0.4*	*0.5*	*2.8*	*0.4*	*0.3*	
31	11.0	15.4	8.7	16.3	9.1	9.6-12.4
std. dev.	*0.3*	*0.4*	*1.1*	*0.4*	*0.2*	
38	11.4	12.9	10.0	14.3	9.8	5.4-17.4
std. dev.	*0.4*	*3.6*	*0.6*	*3.8*	*1.8*	
46	11.7	16.0	10.5	17.0	14.6	10.7-12.6
std. dev.	*0.3*	*0.3*	*0.3*	*0.4*	*0.6*	
53	12.4	16.5	12.1	17.7	15.3	10.6-14.1
std. dev.	*0.3*	*0.7*	*1.1*	*0.6*	*0.7*	
60	12.0	16.2	13.7	17.2	15.6	10.0-14.0
std. dev.	*0.2*	*0.4*	*1.7*	*0.4*	*0.5*	
69	12.4	16.2	16.3	17.2	15.8	11.5-13.4
std. dev.	*0.2*	*0.3*	*0.3*	*0.4*	*0.6*	

Table 24. Culture dilutions (50% sample concentrations) for sample D: organic/inorganic components and boiling pretreated sample (Experiments 5D1 and 5D2).

	PERCENT METHANE IN HEADSPACE						
DAY	CONTROL	PHENOL CONTROL	50% SAMPLE	ORG. PHASE	INORG. PHASE	HIGH TEMP. PRETR.	NON-SIGNIF. RANGE(P<0.05)
3	5.4	5.3	1.0	3.9	1.3	4.3	4.8-5.9
std. dev.	*0.4*	*0.1*	*0.1*	*0.2*	*0.1*	*0.1*	
7	7.1	7.3	1.5	6.8	2.1	6.2	6.3-7.9
std. dev.	*0.5*	*0.1*	*0.2*	*0.3*	*0.1*	*0.4*	
10	8.3	8.1	2.1	8.3	2.5		7.6-8.9
std. dev.	*0.5*	*1.2*	*0.2*	*0.2*	*0.1*		
16	9.1	12.8	2.2	11.6	3.0	8.6	7.9-10.3
std. dev.	*0.3*	*1.1*	*0.1*	*0.7*	*0.6*	*0.6*	
23	10.4	15.0	2.6	12.0	3.9	12.1	5.2-15.5
std. dev.	*0.4*	*0.5*	*0.2*	*4.4*	*1.6*	*0.5*	
31	11.0	15.4	2.7	15.9	4.6	12.7	8.2-13.8
std. dev.	*0.3*	*0.4*	*0.3*	*0.1*	*2.5*	*0.4*	
38	11.4	12.9	3.3	16.2	5.2	12.8	7.7-15.1
std. dev.	*0.4*	*3.6*	*0.7*	*0.5*	*3.2*	*0.8*	
46	11.7	16.0	4.1	16.5	5.5	13.7	7.2-16.2
std. dev.	*0.3*	*0.3*	*1.9*	*0.1*	*3.7*	*0.4*	
53	12.4	16.5	4.8	16.6	6.2	14.2	6.8-18.0
std. dev.	*0.3*	*0.7*	*2.7*	*0.9*	*4.3*	*0.5*	
60	12.0	16.2	5.4	16.6	7.4	14.0	4.3-19.7
std. dev.	*0.2*	*0.4*	*2.4*	*0.1*	*6.7*	*0.4*	
69	12.4	16.2	6.3	16.7	7.6	13.2	4.3-20.6
std. dev.	*0.2*	*0.3*	*2.6*	*0.1*	*6.9*	*1.5*	

Table 25. Boiling pretreated sample A culture dilutions (Experiment 6A1).

	PERCENT METHANE IN HEADSPACE				
DAY	CONTROL	PHENOL CONTROL	30% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.0	0.8	0.5	0.2	0.7-1.3
std. dev.	*0.2*	*0.1*	*0.1*	*0.1*	
4	2.7	3.1	3.1	1.2	2.2-3.1
std. dev.	*0.3*	*0.1*	*0.2*	*0.1*	
10	3.6	4.9	4.4	1.5	2.8-4.4
std. dev.	*0.6*	*0.1*	*0.3*	*0.1*	
18	4.9	8.2	7.8	3.4	3.6-6.2
std. dev.	*0.6*	*0.8*	*0.3*	*0.3*	
25	5.5	12.7	8.4	6.6	3.8-7.1
std. dev.	*0.5*	*1.1*	*0.2*	*0.6*	
32	5.8	14.2	8.8	9.7	4.6-6.9
std. dev.	*0.5*	*0.4*	*0.2*	*0.7*	
40	6.0	14.6	9.0	9.6	4.5-7.4
std. dev.	*1.0*	*0.3*	*0.6*	*0.2*	
47	5.9	14.1	9.4	10.4	3.1-8.6
std. dev.	*0.6*	*1.7*	*0.9*	*1.1*	
54	6.6	14.4	9.6	10.4	5.6-7.6
std. dev.	*0.6*	*0.4*	*0.4*	*0.4*	
63	6.7	14.6	9.9	10.8	5.7-7.8
std. dev.	*0.5*	*0.2*	*0.3*	*0.6*	
71	7.3	15.3	10.7	11.3	6.2-8.4
std. dev.	*0.5*	*0.3*	*0.3*	*0.6*	

Table 26. Boiling pretreated sample B culture dilutions (Experiment 681).

DAY	PERCENT METHANE IN HEADSPACE				
	CONTROL	PHENOL CONTROL	30% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.0	0.8	1.7	1.8	0.5-1.5
std. dev.	*0.2*	*0.1*	*0.2*	*0.1*	
4	2.7	3.1	3.8	3.9	2.0-3.3
std. dev.	*0.3*	*0.1*	*0.5*	*0.2*	
10	3.6	4.9	5.0	5.4	2.8-4.4
std. dev.	*0.6*	*0.1*	*0.7*	*0.1*	
18	4.9	8.2	6.1	7.1	3.4-6.2
std. dev.	*0.6*	*0.8*	*0.6*	*0.1*	
25	5.5	12.7	6.4	7.8	4.1-6.8
std. dev.	*0.5*	*1.1*	*0.6*	*0.1*	
32	5.8	14.2	6.7	7.8	4.3-7.3
std. dev.	*0.5*	*0.4*	*0.5*	*0.2*	
40	6.0	14.6	6.2	8.2	3.7-8.3
std. dev.	*1.0*	*0.3*	*0.4*	*0.3*	
47	5.9	14.1	5.6	8.6	2.4-9.3
std. dev.	*0.6*	*1.7*	*1.7*	*0.5*	
54	6.6	14.4	7.1	8.2	4.3-8.9
std. dev.	*0.6*	*0.4*	*1.0*	*0.3*	
63	6.7	14.6	7.4	8.3	5.4-8.1
std. dev.	*0.5*	*0.2*	*1.1*	*0.3*	
71	7.3	15.3	7.9	8.7	6.0-8.6
std. dev.	*0.5*	*0.3*	*1.2*	*0.4*	

Table 27. Boiling pretreated sample C culture dilutions (Experiment 6C1).

DAY	PERCENT METHANE IN HEADSPACE				
	CONTROL	PHENOL CONTROL	30% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.0	0.8	0.9	0.8	0.7-1.3
std. dev.	*0.2*	*0.1*	*0.1*	*0.1*	
4	2.7	3.1	2.2	2.8	0.8-4.5
std. dev.	*0.3*	*0.1*	*1.2*	*0.4*	
10	3.6	4.9	3.9	3.7	2.8-4.4
std. dev.	*0.6*	*0.1*	*0.2*	*0.1*	
18	4.9	8.2	4.5	4.3	4.0-5.7
std. dev.	*0.6*	*0.8*	*0.1*	*0.1*	
25	5.5	12.7	4.8	4.4	4.6-6.3
std. dev.	*0.5*	*1.1*	*0.2*	*0.1*	
32	5.8	14.2	5.1	4.4	4.9-6.6
std. dev.	*0.5*	*0.4*	*0.4*	*0.1*	
40	6.0	14.6	5.3	4.4	4.5-7.5
std. dev.	*1.0*	*0.3*	*0.4*	*0.2*	
47	5.9	14.1	5.3	4.5	4.8-6.9
std. dev.	*0.6*	*1.7*	*0.3*	*0.3*	
54	6.6	14.4	6.2	4.4	5.7-7.6
std. dev.	*0.6*	*0.4*	*0.3*	*0.4*	
63	6.7	14.6	6.5	4.5	5.7-7.8
std. dev.	*0.5*	*0.2*	*0.2*	*0.5*	
71	7.3	15.3	7.1	4.8	6.2-8.4
std. dev.	*0.5*	*0.3*	*0.2*	*0.6*	

Table 28. Boiling pretreated sample D culture dilutions (Experiment 6D1).

DAY	PERCENT METHANE IN HEADSPACE CONTROL	PHENOL CONTROL	30% SAMPLE	50% SAMPLE	NON-SIGNIF. RANGE (P<0.05)
1	1.0	0.8	0.9	1.0	0.5-1.5
std. dev.	*0.2*	*0.1*	*0.2*	*0.1*	
4	2.7	3.1	2.4	2.0	2.0-3.3
std. dev.	*0.3*	*0.1*	*0.2*	*0.3*	
10	3.6	4.9	3.1	2.1	2.5-4.6
std. dev.	*0.6*	*0.1*	*0.2*	0.5	
18	4.9	8.2	4.9	2.6	3.8-5.9
std. dev.	*0.6*	*0.8*	*0.2*	*0.5*	
25	5.5	12.7	5.8	3.0	4.1-6.8
std. dev.	0.5	*1.1*	*0.6*	*0.6*	
32	5.8	14.2	6.2	3.6	4.3-7.3
std. dev.	*0.5*	*0.4*	*0.6*	*0.8*	
40	6.0	14.6	6.2	4.7	3.7-8.3
std. dev.	*1.0*	*0.3*	*0.6*	*1.3*	
47	5.9	14.1	6.2	5.8	2.4-9.3
std. dev.	*0.6*	*1.7*	*1.5*	*2.0*	
54	6.6	14.4	6.6	6.5	4.3-8.9
std. dev.	*0.6*	*0.4*	*0.7*	*1.4*	
63	6.7	14.6	6.9	7.5	5.4-8.1
std. dev.	*0.5*	*0.2*	*0.7*	*0.5*	
71	7.3	15.3	7.5	8.1	6.0-8.6
std. dev.	*0.5*	*0.3*	*0.8*	*0.4*	